Energy Regulating Hormones and the Development of Obesity and Diabetes

By

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ABSTRACT

Obesity, a complex multifactorial disease, is a serious public health concern due to numerous obesity-related comorbidities thought to result from various hormones in combination with lifestyle and environmental factors. At present, the role of only a fraction of the hormones involved in obesity have been well defined. The aims of this thesis were to investigate: 1) the association of appetite regulating hormones with obesity and obesity-related phenotypes in the Newfoundland and Labrador general population; 2) the response and interaction of appetite and energy regulating hormones on obesity development under a positive energy challenge; 3) the association of dietary magnesium intake with obesity and diabetes; 4) testing the validity of the body adiposity index (BAI) in a Caucasian population; 5) the association of BAI with cardiometabolic risk factors (CRF); 6) and the development of a novel and more accurate equation than body mass index (BMI)and BAI to evaluate adiposity. All of these were achieved using data from two different studies - the large scale, population-based CODING (Complex Diseases in the Newfoundland Population: Environment and Genetics) study and an intervention-based, 7-day positive energy surplus study.

We have discovered that circulating PYY (appetite suppressant) is not significantly associated with obesity status defined by either percent body fat (%BF) or BMI. However, circulating PYY was influenced by age, smoking, medication use, and menopausal status in women. We also sought to explore the endocrine response of normal-weight, overweight and obese individuals to a 7-day hypercaloric diet. We

demonstrated that PYY increased in response to overfeeding, but was not associated with changes in insulin resistance and/or weight. We also found that adiponectin (insulin sensitizer) was not associated with obesity status but the significant increase in adiponectin, due to overfeeding, was significantly associated with insulin resistance.

We also investigated the impact of dietary magnesium on obesity and obesity-related co-morbidities and found that higher dietary magnesium intake is associated with improved insulin sensitivity and this effect is particularly beneficial for overweight and obese individuals in the general population along with pre-menopausal women. Therefore, our data would suggest that the beneficial effects of magnesium intake are less sensitive among post-menopausal women.

In addition, we explored the applicability of the BAI and found that it was a better estimate of adiposity than BMI in the non-obese Caucasian population. The BAI was also more closely associated with the relationship of %BF with CRFs than BMI. However, this association is significantly attenuated when assessing the influence of increasing BAI on CRFs amongst men and women examined separately.

Lastly, we developed a sex-specific equation to overcome the gender related weakness of both BMI and BAI to predict adiposity. The body fat index (BFI) sex-specific equations we developed more accurately predict %BF than BMI and BAI in the general population and at various extremes of adiposity in men and women.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Full name
%AF	Android fat percentage
%BF	Body fat percentage
%GF	Gynoid fat Percentage
%TF	Trunk fat Percentage
ADP	Air displacement plethysmography
AgRP	Agouti-related protein
ANOVA	Analysis of variance
ANCOVA	Analysis of covariance
BAI	Body adiposity index
BFI	Body Fat Index
BIA	Bioelectrical impedance analysis
BMI	Body mass index
CART	Cocaine- and amphetamine-regulated transcript
CCK	Cholecystokinin
CDA	Canadian diabetes association
CNS	Central nervous system
CODING	Complex Diseases in the Newfoundland Population: Environment and Genetics
CRF	Cardiometabolic Risk Factors
CRH	Corticotropin-releasing hormone
DPP-IV	Dipeptidyl peptidase-IV

DXA	Dual-energy X-ray absorptiometry
ELISA	Enzyme-linked immunosorbent assay
FFQ	Food frequency questionnaire
FTO	Fat mass and obesity-associated gene
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GWAS	Genome-wide association study
HDL-c	High-density lipoprotein cholesterol
HOMA	Homeostatic model assessment
HOMA-β	Homeostatic model assessment-β-cell function
HOMA-IR	Homeostatic model assessment-insulin resistance
HREA	Health research ethics authority
IL-6	Interluekin-6
LDL-c	Low-density lipoprotein cholesterol
MC4R	Melanocortin receptor 4
mRNA	Messenger RNA
MRI	Magnetic resonance imaging
NL	Newfoundland and Labrador
NPY	Neuropeptide Y
NW	Normal weight
OW	Overweight
OB	Obese
PEC	Positive energy challenge

POMC	Pro-opiomelanocortin
PP	Pancreatic polypeptide
PYY	Peptide tyrosine tyosine (peptide YY)
RBP4	Retinol Binding Protein 4
SD	Standard deviation
T2D	Type 2 diabetes
TRH	Thyrotropin-releasing hormone
TG	Triacylglycerol
TNF-α	Tumor necrosis factor alpha
WAT	White adipose tissue
WHO	World Health Organization

Co-Authorship Statement

This thesis contains seven manuscripts, five of which have been published in peer-reviewed journals. For each manuscript I participated in data collection, data analysis and primary manuscript writing. I had a lead role in all laboratory work including; DXA scanning/ interpretation, plasma/serum isolation, DNA/RNA isolation, along with the measurements of PYY, PP, CCK, GLP-1, Ghrelin, Leptin, Adiponectin, and TNF-α from fasting serum/plasma. I performed the majority of data analyses, participated in the interpretation of all findings and wrote each of the manuscripts included in this dissertation. I am listed as second author for Chapter 6 ("Concordance of BAI and BMI with DXA in the Newfoundland Population"). For this chapter I participated in data collection, did all data analyses, aided in the drafting/writing of manuscript, and submitted/revised the manuscript for final publication.

1

Chapter 1.Introduction

1.1 - Prevalence of Obesity and Obesity-Related Comorbidities

Obesity, defined by excessive fat accumulation, is associated with a number of serious health conditions and has become one of the leading risk factors for global mortality [1]. The World Health Organization (WHO) estimates that, globally, more than 500 million men/women are obese and is projected to grow to over 700 million before the end of the decade[1]. In Canada, the prevalence of obesity has tripled from 1985 to 2011[2]. The high prevalence of obesity and obesity-related comorbities have grown to epidemic proportions culminating in a significant fiscal burden to health systems across the globe [3]. Currently, 34.9% of the United States [4] and 18.3% of Canadians [2] are obese representing an annual health cost of ~$147 billion [5] and ~$7 billion [6], respectively. These fiscal burdens are principally attributable to the mirrored rise in obesity-related chronic health conditions such as: type 2 diabetes, hypertension, cardiovascular disease, and various cancers[7-10]. Newfoundland and Labrador having the highest rates of obesity and diabetes in Canada makes these health conditions especially pertinent to this Canadian province [11, 12]. A report by Statistics Canada in 2011, revealed that 29% and 40% of the Newfoundland and Labrador population were obese and overweight, respectively [13]. Currently, 69% of Newfoundlanders and Labradorians are overweight/obese [12] and approximately 10% of the general population struggle with diabetes [11], which corresponds to an annual cost of $254 million dollars. Data from the Canadian Community Health Survey (CCHS) and the Canadian Diabetes Association (CDA) project that by the year 2020 over 71% of Newfoundland and Labradorians will be overweight/obese and the prevalence of diabetes

will exceed 15%[11, 12]. Although a number of methods are used to determine whether an individual is normal-weight, overweight or obese; the most widely implemented is the body mass index (BMI). The body mass index indirectly estimates adiposity by dividing a body weight in kilograms by the square of height in meters [kg/m^2]. According to the World Health Organization, a BMI $\geq$ 25 kg/m^2 is classified as overweight, while a BMI $\geq$ of 30 kg/m^2 is classified as obese. However, BMI often mis-classifies obesity status up to two obesity categories due to its inability to distinguish fat from muscle and bone [14-17]. More importantly, even though BMI has been heavily criticized for not accurately representing adiposity or defining obesity, the majority of epidemiology studies still utilize BMI due to accessibility, low cost, and simplicity. Therefore, caution should be exercised whenever BMI is being utilized to establish obesity. In fact World Health Organization now officially recognizes the issue [18] of false-negative classification of obesity status when defined by BMI.

1.2 - Etiology of Obesity

Obesity is complex with a multifaceted etiology. Generally, obesity is the result of weight gain due to a chronic positive caloric balance where the total daily energy intake is greater than the total daily energy expenditure. Although a lack of physical activity and/or the increased accessibility/consumption of calorie dense foods are the primary contributing factors to obesity, genetics, diet composition and the environment also play important roles in energy regulation and body weight maintenance. For example, the *ob/ob* mouse is deficient in leptin which is an energy regulating hormone

acting on the hypothalamus to regulate energy expenditure and eating behavior[19]. In addition, the administration of leptin to these mice ameliorated the reduction in energy expenditure and excessive food intake [20]. A study by Montague and Farooqi et al. [21] examined two severely obese children finding very low circulating leptin levels and found an homozygous frame-shift mutation where a single guanine nucleotide in codon 133 of the leptin gene was deleted. A follow up study by Farooqi et al. also revealed that the administration of the recombinant methionyl leptin increased energy expenditure and significantly reduced food intake and body fat. These studies provided the first genetic evidence that leptin, an energy regulator adipose tissue derived cytokine, was an important player in body fat management. A study by Frayling et al. [22] discovered that a common variant of the FTO gene was significantly associated with BMI in children and adults. The study found that adults who were homozygous for the risk allele weighted about 3 kilograms heavier. They also found that adults homozygous for the risk allele had 1.67 greater odds of being obese compared to those without the risk allele. Lastly, twin studies have also provided strong evidence that genetics plays an important role in obesity development [23-26]. In addition, twin studies have also revealed the significant influence of the environment on obesity development [24, 25]. Therefore, genetic and/or environmental disruptions can significantly affect the optimal performance of various energy regulatory pathways resulting in the increased susceptibility to weight gain leading to obesity and obesity-related conditions. Therefore, endocrinology also has a significant influence on energy homeostasis, via hormones playing an integral role in appetite regulation and glucose/lipid metabolism. For example, some of the energy

regulating hormones which enter circulation can act directly on the CNS and influence eating behavior [27, 28]. Peptide YY (PYY), released into circulation from the gastrointestinal tract, is one such hormone which can cross the blood brain barrier and act directly upon the hypothalamus to reduce appetite [29]. In addition, the adipose tissue derived cytokine adiponectin and leptin, when released into circulation, can also significantly affect insulin sensitivity, energy expenditure, and eating behavior. Due to the significant influence of energy regulating hormones on metabolism and their potential role in the development of adiposity and adiposity-related health conditions we chose to investigate a number of such hormones suspected in the development of obesity/diabetes throughout my PhD work. In addition, since BMI is a very weak predictor of adiposity we utilized a much higher resolution measurement of body fat via a Dual-Energy X-ray Absorptiometry (DXA) machine. A DXA system can very accurately distinguish fat mass from fat-free mass which allowed me to evaluate the direct association of various gastrointestinal (PYY, GLP-1, and ghrelin) and adipose tissue derived cytokines (Adiponectin) with adiposity. The DXA system also provided me the ability to directly explore the impact of nutrition on the development of adiposity and obesity-related conditions while also designing a new sex specific predictive adiposity equation for epidemiological researchers wishing to more accurately define and investigate human obesity at the population level.

1.3 - Central Nervous System, Gastrointestinal Hormones and Appetite Regulation

Appetite, a key factor in energy homeostasis, is multifaceted and significantly influences the regulation of body weight through both neural and hormonal signals in the central nervous system (CNS) [30, 31]. Our brain is responsible for receiving and interpreting orexigenic/anorexigenic signals that can alter both behaviour and metabolic processes [31]. Specifically, the gastrointestinal tract is one of the largest endocrine organs in the human body secreting several appetite regulating hormones. The brainstem and hypothalamus receive both neural and hormonal signals through a gut-brain communication pathway subsequently regulating food intake and energy homeostasis[31]. Since gut hormones influence appetite and play an integral role in glucose/lipid metabolism and insulin sensitivity through gut-brain communication [27, 28]understanding the relationship between appetite regulating hormones and adiposity may provide valuable insight into the underlying mechanisms responsible for the development of obesity.

The hypothalamus, which coordinates many neuroendocrine inputs, is the primary control center for appetite. The neurons critical to energy regulation and appetite located within the hypothalamus are the arcuate, paraventricular and ventromedial nuclei[31] which each contain a unique population of neurons. Many gastrointestinal hormones like PYY, GLP-1 and ghrelin can freely cross over the blood brain barrier to exert a direct influence on neurons within the hypothalamus [31]. The arcuate nucleus neurons are the

most frequently studied and well known regarding appetite regulation. The orexigenic, appetite stimulating, neurons of the arcuate nucleus are the neuropeptide Y (NPY) and agouti-related protein (AgRP) producing neurons [32-34]. The activation of either the NPY or the AgRP nuclei will result in the increase of food intake and a reduction in energy expenditure [35, 36]. For example ghrelin, an appetite stimulating hormone, binds to the growth hormone secretagogue receptor (GHS-R) in the arcuate nucleus to increase food intake [37, 38]. The anorexigenic, appetite suppressing, neurons found within the arcuate nucleus are the cocaine- and amphetamine-regulated transcript (CART) and proopiomelanocortin (POMC) neurons. The stimulation of either the CART or POMC will result in a decrease in food intake and the increase in energy expenditure [35, 36]. For example, the alpha-melanocyte-stimulating hormone (a-MSH) is a derivative of the POMC polypeptide which binds to melanocortin receptor 4 (MC4R) in the paraventricular nucleus reducing food intake and increasing weight loss [39]. In fact, 6% of severe early-onset obesity can be attributed to MC4R mutations [40] which also makes it the most common obesity syndrome described to date, having a prevalence higher than cystic fibrosis [41].

The paraventricular nucleus contains neurons which synthesize and release corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) influence energy expenditure and appetite [42]. For example, both the CRH and TRH, anorexigenic neuropeptides synthesis and secretion is significantly reduced in a fasted

state [43, 44]. Paraventricular nucleus neurons also have many projections from the arcuate nucleus influencing food intake.

The dorsomedial nucleus has a large number of NPY projections which influences appetite. For illustration, α-MSH stimulation results in the activation of the dorsomedial nucleus neurons which in turn reduces food intake [45]and damage to the area of the hypothalamus results in overeating and weight gain [46]. In addition, studies have shown that the administration of Cholecystokinin (CCK), an appetite suppressant, reduces NPY activation in the dorsomedial nucleus which in turn inhibits food intake [47]. The ventromedial nucleus neurons are significantly influenced by α-MSH and receives neuronal projections from the arcuate nucleus [48]. Case in point, the MC4 receptor is very prevalent in the ventromedial nucleus and the administration of α-MSH significantly increases food intake in rodents [48].

The lateral hypothalamus is best known for extensive MSH terminals and the synthesis and release of the melanin-concentrating hormone (MCH) [49]. MCH, a 13 amino acid appetite stimulating hypothalamic hormone, is a very important energy regulating peptide which is also involved in skin pigmentation. For instance, MCH has been found to increase in the fasted state and its administration is found to increase feeding and weight gain [50]. Since obesity is one of the fastest growing health conditions worldwide and the underlying mechanisms of food intake and energy homeostasis are playing a significant role, investigating the hormones which directly

influence appetite is important. Since gastrointestinal hormones in circulation can act directly on the hypothalamus to affect eating behavior, our laboratory and I set out to investigate the influence of PYY, GLP-1 and ghrelin on the development of obesity and obesity-related comorbidities.

1.3.1 - Peptide Tyrosine Tyrosine (PYY)

Peptide YY (PYY), a 36 amino acid appetite suppressing gut hormone, is secreted from the L-cells of the gastrointestinal tract which acts centrally on the hypothalamus to inhibit the orexigenic activity of the neuropeptide Y (NPY)[30, 51, 52]. PYY is released from the mucosa in the ileum and colon of the gastrointestinal tract existing in a PYY_{3-36} and PYY_{1-36} form [53, 54]. PYY secretion increases in response to food consumption and is positively correlated with the calorie content of meals [55-57]. Both forms are physiologically active decreasing pancreatic secretions and suppressing gastrointestinal motility [58]. Circulating PYY increases satiety, decreasing food intake via gut-brain communication and PYY plays an integral part in energy homeostasis [53, 54,59]. PYY is cleaved within the peripheral circulation at the N-terminus by circulating dipeptidyl peptidase IV (DPP-IV), which results in the PYY_{3-36} form. PYY binds to the Y1, Y2, and Y5 receptors, although the PYY_{3-36} form has the highest affinity with the Y2 receptor highly expressed on arcuate NPY neurons [29]. Considering that the activation of the NPY neurons stimulates food intake, the anorexigenic effect of PYY is primarily thought to be mediated by the inhibition of NPY neurons through the Y2 receptor in the arcuate nucleus[30, 53,60]. Data from animal experiments indicate that endogenous PYY acts on

the arcuate nuclei through the gut-hypothalamic pathway blocking various orexigenic effects which results in increased satiety and decreased gastric emptying [30, 53, 60]. It was originally suggested that circulating PYY concentrations were lower in obese rodents as well as humans and that PYY infusion effectively reduces food intake and body weight [30, 51, 53, 60]. The initial studies on the potential role of PYY in obesity development, which were conducted on rodents, revealed that a diet-induced obesity would decrease circulating PYY [30, 61,62]. This would suggest that the circulating concentration of this appetite suppressing gut hormone may be linked with the development of obesity [30, 61,62]. The initial PYY gene knockout studies in mice revealed significant hyperphagia and that the acute administration of PYY could significantly ameliorate this condition [30, 53,61]. This group also proposed that endogenous PYY concentration was lower in obese rodents and humans, inversely associated with obesity-related phenotypes[51], and the administration of PYY could effectively reduce food intake and body weight independent of obesity status [30, 51, 53]. Therefore it was hypothesized that a deficiency in circulating PYY could be a significant contributing factor toward the chronic over consumption of food and subsequent weight gain. However, many successive PYY knockout [63, 64] studies have failed to reproduce the strong association of PYY with hyperphegia and diet-induced obesity. A considerable number of studies have also been unable to replicate the original results [65, 66] along with demonstrating any significant association between circulating PYY and adiposity [58].

The initial human based studies on PYY also found circulating levels to be lower in obesity and inversely correlated with body mass index (BMI), suggesting that PYY deficiency may contribute to human obesity [51, 61, 67, 68]. The early studies found that the infusion of PYY decreased the appetite of men/women subjects along with a subsequent reduction in food intake [51, 53]. Taken together, these data suggest that individuals with a lower concentration of PYY, such as overweight/obese, may have a weaker satiety signal subsequently leading to the over consumption of food and weight gain in humans. However, like in rodents, many subsequent human studies with larger sample sizes could not replicate the relationship between PYY and obesity [69-71]. The administration of PYY was also found not to have any effect on appetite or weight loss [66, 71]. Additionally, an extensive review in 2005,exploring the anorexigenic effect of PYY, revealed 84% studies produced among 41 independent research groups were unable to reaffirm the claim proposed by the initial investigations [69]. Therefore, although PYY was thought to be involved in the development of obesity [51, 53], significant controversy exists regarding the definitive role that PYY plays in this regard [58, 66, 69-71].

The inconsistency in PYY and its association with obesity, we believe, is primarily due to the utilization of modalities which cannot accurately measure adiposity or assess obesity like BMI. Our laboratory [15-17] has shown that BMI cannot accurately distinguish fat mass from fat-free mass and is not an accurate predictor of

adiposity, suggesting that the inaccurate measurement of adiposity and the misclassifications of obesity status could be the factor attributing to the contradictory reports concerning the association of PYY with obesity. Other potential reasons for the contradiction in reports for the association of PYY with adiposity may be gender and/or lifestyle related. A study by Sandstrom et al [72] found that circulating PYY was higher in men compared to women, while another study found that PYY levels are higher in women [70]. Additionally, various studies have proposed that nicotine and [73-75] and medications [76-78] can significantly influence PYY concentration. The involvement of PYY in the development of human obesity is unclear [66, 79, 80], the relationship between circulating PYY and adiposity/obesity-status is controversial [69], and very little data exists from large human population-based studies to help resolve the controversy.

Since few large cross-sectional studies, taking into consideration potential confounding factors and that the current results in the literature are controversial, there is a good reason to explore the influence of circulating PYY on adiposity and obesity-status at the population level. Therefore the purpose of my first study, described in **Chapter 2**, was to investigate the association of circulating PYY concentration with adiposity measured by dual-energy x-ray absorptiometry, in a large population adjusting for major confounding factors. The detailed objectives were to: 1) test if PYY is associated with obesity status by comparing fasting serum PYY between normal-weight (NW), overweight (OW) and obese (OB) men and women; 2) examine the association of PYY with body composition among normal-weight (NW), overweight (OW) and obese (OB) men and women; and 3) determine the influence of age, sex, smoking, medication use

and menopausal status on circulating PYY.

1.3.1.1 - Peptide Tyrosine Tyrosine (PYY) And Positive Energy Balance

Body weight is primarily maintained by the balance between energy intake and energy expenditure. The disruption of this highly regulated energy balance system by a chronic positive energy surplus, leading to the accumulation of adipose tissue, allows researchers the ability to investigate the mechanisms which facilitate and/or attenuate weight gain. Sims et al. [81] was among one the first to explore the effect of an positive energy surplus on humans. These studies had participants increase food intake 300% (~8,000-10,000 kilocalories/day) for a 10-week period of time [81, 82]. Their investigation demonstrated a significant increase in adipose tissue and various hormones. Upon the completion of the intervention, participants experienced a minimum weight gain of 15% of their baseline weight with an average weight gain of 16.4 kg. Another very important human overfeeding study was completed by Bouchard et al. [83] in 1990 published in the New England Journal of Medicine. This investigation overfed 24 participants for 84 days with an energy surplus of 1000 kcal per day which equaled an excess of 84,000 kcal [83]. The novelty of this study was that it was performed on 12 pairs of twins. Upon the completion of the intervention the participants' minimum weight gain was 4.3 kg with an average weight gain of 8.1 kg. Another study on overfeeding (50% greater than normal caloric intake) by Brons et al. [84] 26 normal-weight young men observed a significant increase in both glucose and insulin along with adiponectin, leptin and gastric inhibitory peptide (GIP). They also reported that insulin

secretion preceded the development of insulin resistance. Although, the overfeeding stimulus was strong enough to affect the endogenous concentration of various hormones, the positive energy surplus was not long enough to evoke significant weight gain. Another study by Astrand et al. [85] discovered that after a 4-week positive energy challenge body weight significantly increased 9.5%. The 18 young normal-weight adults (12 male and 6 female) also had elevated concentrations of insulin but resistin and adiponectin remained unchanged. Due to the fact that obesity is generally understood to be the result of a chronic energy surplus where energy intake exceeds energy expenditure [86]which is triggered by many complex hormonal changes [87, 88], I also chose to investigate PYY under these conditions to elucidate its potential contribution to the development of obesity. Previous studies having investigated the role of PYY during the disruption of energy balance have only examined the response of this gut hormone during a negative energy balance. Essah et al [89] demonstrated that an 8-wk low-fat and low-carbohydrate hypocaloric diet on obese participants significantly reduced body weight by 9% along with a 10% reduction in circulating PYY. It would seem that during periods of food intake reduction that the appetite suppressing effects of PYY are reduced to potentially increase food intake. At the present time there is no data regarding the effect of weight gain, through a chronic energy surplus, on PYY or the potential differential effect of obesity status. Our laboratory, [90-92] and others [57, 83-85,93], have demonstrated that changes in nutrition through overfeeding can have major effects on energy regulating hormones. The response of PYY to our short term positive energy will provide valuable insight regarding the potential role of PYY in the development of

obesity as well as other related phenotypes including insulin resistance and serum lipid profiles. Therefore the primary objective of the study, thoroughly described in **Chapter 3**, was to investigate whether PYY levels are affected by a short-term positive energy challenge and if obesity status (normal weight, overweight and obese) influences this response.

1.3.2 - Glucagon-Like Peptide 1 (GLP-1)

Glucagon-like peptide-1 (GLP-1), a 30 amino acid appetite suppressing hormone, is secreted from the L-cells of the distal gastrointestinal tract which acts through the hypothalamus to inhibit orexigenic activity [94]. GLP-1 also acts peripherally to reduce gastric motility, the secretion of glucagon and various gastrointestinal secretions[95, 96]. The active form of GLP-1 is produced from the posttranslational modification of the proglucagon gene product by prohormonecovertase [94]and binds to the GLP-1 receptor (GLP1R) which acts both centrally and peripherally [97]. Although, GLP-1 is known to influence appetite it has also been found to facilitate glucose-dependent insulin secretion [98]. In addition, treatment with GLP-1 or GLP-1 receptor agonists are being investigated as treatments for diabetes and appetite regulation among obese [99, 100]. During my PhD work Danny Wadden and I investigated the association of GLP-1 with body composition, obesity status and obesity-related biomarkers before and after overfeeding (the intake of 70% more calories for 7-days). Our manuscript entitled "Circulating glucagon-like peptide-1 increases in response to short-term overfeeding in men" published in the Journal of Nutrition & Metabolism (DOI:10.1186/1743-7075-10-

33) discovered that circulating GLP-1 is not associated with adiposity at baseline, but GLP-1 significantly increased after a short-term positive energy challenge independent of obesity status. Since GLP-1 is an appetite suppressant, the up regulation during overfeeding may be acting as a protective mechanism to reduce appetite and subsequent weight gain. My work on GLP-1 will not be further discussed throughout this document as this work was used for Mr. Danny Wadden's Master's of Science (MSc) thesis.

1.3.3 - Ghrelin

Ghrelin, the only gastrointestinal appetite stimulating hormone, is a 28-amino acid peptide secreted from the stomach lining which binds to the growth hormone secretagogue receptor (GHS-R) [37]. Ghrelin stimulates hunger and increases food intake and weight gain [101]. Like PYY, ghrelin acts directly on the CNS via the arcuate nucleus of the hypothalamus [102]. However unlike PYY, the administration of ghrelin results in the stimulation of the orexigenic NPY neurons in arcuate nucleus increasing appetite[103-106]. Since the peripheral administration of ghrelin stimulates hypothalamic NPY mRNA expression in the arcuate neurons the orexigenic effect of ghrelin is mediated predominantly through these neurons [107]. In addition, when growth hormone secretagogue receptor for ghrelin is knockout in mice food intake is reduced and weight loss is observed [108].

During my PhD work Danny Wadden and I investigated the association of circulating ghrelin with body composition, obesity status and obesity-related biomarkers before and after overfeeding. Our manuscript entitled "Serum Acylated Ghrelin

Concentrations in Response to Short-Term Overfeeding in Normal Weight, Overweight, and Obese Men" published in the PLoS One Journal (DOI: 0.1371/journal.pone.0045748) discovered that there was no significant difference in ghrelin concentration among normal weight, overweight, and obese young men. Additionally, to our surprise, fasting circulating ghrelin significantly increased in response to our 7-day positive energy challenge. However, although ghrelin was not associated with adiposity, baseline ghrelin was negatively associated with baseline BMI and the change in BMI due to overfeeding. In addition, during my PhD work Peyvand Amini and I investigated the association of ghrelin and PYY with bone density in the general population of Newfoundland and Labrador. Our cross sectional study entitled "Beneficial Association of Serum Ghrelin and Peptide YY with Bone Mineral Density in the Newfoundland Population" published in BMC Endocrine Disorders discovered that there is a positive association of ghrelin with bone density in women independent of BMI, physical activity, age, alcohol consumption, and smoking. However, PYY was not associated with bone density in the Newfoundland population.

Lastly, during my PhD work Peyvand Amini, Danny Wadden and I also investigated the association of ghrelin with body composition, obesity status and obesity-related biomarkers in the general population of Newfoundland and Labrador. Our manuscript entitled "Serum Acylated Ghrelin Is Negatively Correlated with Insulin Resistance In the CODING study" published in PLoS One (DOI: 10.1371/journal.pone.0045657) discovered that high circulating levels of ghrelin are associated with low levels of insulin resistance in the Newfoundland population. Ghrelin

was significantly negatively associated with circulating insulin level and measurements of insulin resistance in the general population and among men and women separately. However, ghrelin concentration was not associated with adiposity. My work on ghrelin will not be further discussed throughout this document as the above work was used for the Masters of Science (MSc) thesis for Mr. Danny Wadden and Dr. Peyvand Amini.

1.4 - Adiponectin and Obesity

Adiponectin, an insulin sensitizing and anti-inflammatory hormone, is a 244 amino acid peptide secreted primarily from adipose tissue and binds to the ADIPO1 and ADIPO2 receptors [109]. White adipose tissue (WAT), once only regarded as a site for energy storage, is now known to play an integral role in human energy homeostasis [110-112]secreting a large number of physiologically active proteins [113-115]. Specifically, adiponectin has been shown to attenuate insulin resistance and effectively increase the disposal of circulating glucose [116-118]. Considering that adiponectin has been recognized as a significant insulin sensitizer it would be consistent to anticipate that circulating adiponectin levels would be considerable in the presence of an insulin resistant state such as obesity. However, the puzzling actuality suggests that adiponectin is inversely associated with obesity [119-124] and insulin resistance [122, 125-127]. The majority of human cross-sectional studies show that circulating adiponectin is diminished among the obese population [119, 122-124], and significantly promoted after weight reduction [124, 125, 128, 129]. Since white adipose tissue is the primary location for the adiponectin production and secretion it would seem logical to assume that circulating

adiponectin may be regulated by adiposity feedback inhibition [124]. Although, evidence suggests that adiponectin is strongly associated with glucose and lipid metabolism, the role of adiponectin in the development of obesity and insulin resistance remains unclear. The ob/ob obese mouse model has shown that adiponectin secretion is significant reduced [127, 130, 131], however the adiponectin transgenic ob/ob mice can effectively ameliorate insulin resistance but not obesity [132, 133]. In fact, adiponectin knockout mice have been shown to severely attenuate glucose disposal and facilitate insulin resistance without affecting weight or adiposity [126, 134]. Additionally, the administration of adiponectin to ob/ob, db/db, and adiponectin knockout mice can ameliorate the development of insulin resistance without significantly influencing adiposity [127, 135]. It would seem that adiponectin may not be mediated by adiposity, but rather the increase in insulin insensitivity. Since, body weight regulation interventions decreasing excess body fat have been shown to increase circulating levels of adiponectin and improve insulin resistance [124], lifestyle interventions have become an appealing treatment for insulin resistance potentially due to hypoadiponectinemia found among obese [136]. In addition, hormones like TNF-α have been found to inhibit adiponectin release, while adiponectin has been shown to inhibit TNF-α in adipose tissue [134]. Adiponectin also reduces the expression of adhesion molecules in endothelial cells and elicits its anti-inflammatory properties by decreasing cytokine production from macrophages [137]. Furthermore, it promotes insulin sensitivity and the inhibition of the inflammatory mediators [124]. Esposito et al.[138] showed a significant improvement in adiponectin levels following a comprehensive multidisciplinary approach towards

lifestyle including nutrition guidelines proposed by the "Mediterranean Diet". The study found that reducing body weight in obese women through lifestyle variations such as diet were associated with increases in adiponectin and a reduction in insulin resistance markers. Current human based overfeeding studies are controversial regarding its influence on adiponectin concentrations. A study by Brons et al. [84] found after a 5-day high-fat (60% fat, 32.5% carbohydrates and 7.5% protein) of overfeeding (50% greater calories per/day) that circulating adiponectin increased by 13.4%. They provided the first evidence that up regulation of adiponectin is likely due to significant increase total caloric intake rather than macronutrient composition of the diet. Since the subjects in this study were only normal-weight young men their findings cannot be extrapolated to normal-weight women or overweight and/or obese men and women [84]. However, a 4-week overfeeding study by Astrand et al [85] on 12 male and 6 female normal weight subjects did not find a significant increase in adiponectin concentration. Again, since this study only consisted of normal-weight individuals' assumptions cannot be made about the effect of overfeeding on adiponectin concentration among obese individuals. Lastly, a 100 day overfeeding (840 kcal more calories per day) study by [93] on 12 pairs of male identical twins found that adiponectin concentration actually decreased. In addition, no correlation was found between adiponectin at baseline and adiposity before or after prolonged overfeeding. However, like the previous studies the aforementioned results can only be applied to normal-weight individuals. In addition, recent studies have hypothesized that the potential negative association of adiponectin with obesity is in fact more dependent upon the development of insulin resistance [116, 120, 123, 139].

Although some studies have indicated that the reduction in adiponectin is primarily due to a concomitant increase in insulin resistance during obesity [120, 121, 139], no study to date has investigated the potential paradoxical correlation of adiponectin with the increase in adiposity and insulin resistance due to short-term overfeeding. As aforementioned our laboratory and others have demonstrated that a short-term positive energy challenge significantly affects glucose and lipid metabolism, which results in significant body weight gain and insulin resistance [83-85, 90-92]. Moreover, changes in nutritional status have also shown to influence circulating adipokines [83-85, 90,91]. However, to date, very few overfeeding studies have explored the influence of stressor, short term positive energy challenge, which is significant enough to increase both insulin resistance and body fat. The publication described in **Chapter 4** is the first study of its kind to investigate the effect of a 7-day positive challenge (70% above normal energy requirements) on circulating adiponectin among normal-weight, overweight and obese individuals defined by BMI and body fat percent measured by DXA.

1.5 - Dietary Intake of Magnesium and Obesity

Magnesium, a cofactor required in over 300 enzymatic reactions, is the fourth most abundant cation in the human body involved with both glucose metabolism and insulin homeostasis [140, 141]. Magnesium has been proposed to be functionally related to glucose metabolism through an interaction with tyrosine-kinase activity on the insulin receptor which is associated with the development of insulin resistance and type 2 diabetes [142]. More specifically, phosphorylation of the tyrosine kinase enzyme of the

insulin receptor, required for post-receptor insulin sensitivity and subsequent insulin-mediated glucose uptake, are dependent on magnesium [143]. The apparent protective role of magnesium on IR and type 2 diabetes has not been fully explained but is likely due to enhanced insulin sensitivity through multiple mechanisms. Although recent evidence has suggested that dietary magnesium intake may play an important role in enhancing insulin sensitivity, population based studies have found conflicting evidence regarding the potential benefit of dietary magnesium intake. Obesity, as a disorder, can place individuals at a significantly elevated risk for impaired insulin action [144]and various metabolic abnormalities such as hypertension, dyslipidemia, and glucose intolerance [144]. For example, type 2 diabetes comprises 90% of all diabetic cases and has become an ever increasing healthcare challenge as the number of people affected reaches epidemic proportions [1]. The prevalence of this condition is expected to reach over 438 million people globally by the year 2030 and carries with it a significant fiscal burden [145]. The functional relationship between various hormones and obesity-related conditions is a significant focus of my obesity research, however diet composition is also a heavily studied area of work [145, 146]. Although there is currently no medical intervention capable of preventing the development of diabetes, simple lifestyle modifications (such as increased physical activity, moderate weight loss, and eating behavior modifications) have been shown to attenuate the onset of type 2 diabetes [1, 146]. A number of studies revealed a significant correlation of low dietary magnesium intake [147-149] with increased insulin resistance [150-152].

Villegas et al. [151] designed a study to assess the risk of type 2 diabetes in women and found a negative correlation between magnesium intake and type 2 diabetes risk. Guerrero-Romeo et al, [153] found that dietary magnesium intake is positively associated with improvements of insulin resistance. Additionally, studies by Villegas et al. [151] and Kim et al. [150] have shown that dietary magnesium is beneficially associated with markers of insulin resistance and is more pronounced in overweight patients [150, 151]. These studies provide strong evidence that overweight/obese individuals could potentially benefit from an increase in magnesium intake [150, 151]. These findings would suggest that individuals with excessive adiposity may be able to better absorb and metabolize magnesium resulting in enhanced insulin action. Although controversy exists regarding whether dietary magnesium intake can attenuate the development of diabetes [154-156], none of the above studies adequately controlled for adiposity in their analysis. In fact the majority of studies evaluating the association between dietary magnesium and insulin resistance have used BMI when attempting to control for adiposity. Percent body fat and BMI likely represent different physiological entities, and that adiposity is the parameter most closely linked with the development of insulin resistance [157]. It is therefore, critical that accurate measurements of body fat be used when assessing the influence of adiposity on the relationship of magnesium intake with insulin resistance. This is an important point to consider when the relative amount and distribution of adipose tissue, both important determinants of insulin sensitivity, cannot be determined by the BMI [158-160]. Due to the lack of a large cross-sectional population based study systematically controlling for body fat when investigating the

association of dietary magnesium intake on insulin resistance, we designed a study to fill this gap. The manuscript described in **Chapter 5** is the first largest cross-sectional study to systematically control for major confounding factors (age, gender, caloric intake, physical activity, medication use, smoking status, menopause), including body fat percentage, to investigate the influence of dietary magnesium intake on insulin resistance.

1.6 - Measuring Adiposity and Defining Obesity Status

Obesity, defined by the excessive accumulation of adipose tissue, and obesity-related comorbities have grown to epidemic proportions resulting in a critical global health issue [18, 161]. In fact the significant rise in obesity is mirrored by a rise in cardiometabolic risk factors [7-10]. Considering that Newfoundland and Labrador have the highest prevalence of both obesity and diabetes among the Canadian provinces[12, 162], the development of obesity and its comorbidities are especially relevant. However as mentioned earlier in the "*Prevalence of Obesity and Obesity-Related Comorbidities*" section, due to the over usage of BMI to define obesity in previous studies, caution must be taken assuming that the associations found with cardiometabolic risk factors have to do with adipose tissue accumulation. Moreover, since BMI cannot differentiate fat mass from fat-free mass the association of adiposity with health risk factors has come into question [15, 16]. Although, air-displacement plethysmograohy (ADP), underwater weighing, magnetic resonance imaging (MRI) and/or dual-energy X-ray absorptiometry (DXA) are among the most precise measurements of body fat, conversely these methods are both expensive and impractical to assess obesity at the population level [163-165].

1.6.1 - The Body Mass Index (BMI) and Obesity

The body mass index (BMI), calculated as weight divided by height squared ((Weight-kg)/(height-m)^2), was designed over 100 years ago [166] and due the low cost and simplicity of implementation it is the most widely known and commonly used classification measure used by obesity health professionals. BMI is currently the method utilized for the World Health Organization's (WHO) annual reports regarding obesity and also utilized in the majority of population based studies investigating obesity and health risk factors relating to obesity. In fact, the World Health Organization (WHO) still defines obesity as the abnormal or excessive fat accumulation represented by a BMI value greater than or equal to 30 kg/m^2 [18]. Although BMI is the most cost effective method to evaluate adiposity, it has been shown to have significant weaknesses in accurately determining adiposity. The primary weakness of BMI is that it cannot distinguish fat mass from and fat-free mass [15, 16]. Another significant weakness of BMI is that it does not account the sexual dimorphism of adiposity, although gender differences for body fat percentage have been well documented [167-169]. The lack of sex criteria for BMI is a significant limitation to its ability to properly predict body fat. The body mass index also does not account for the variation of accumulation of body fat with age nor the inter-individual difference of body fat percentage among people within the same BMI category [15]. Considering that studies by our laboratory, and others, have shown that a BMI score can mis-classify by one or even two obesity categories [14, 15] caution must be exercised when utilizing BMI for obesity research. In fact, due to the

abundance of data strongly supporting the weakness of BMI in predicting adiposity, the WHO now admits that caution must be exercised when utilizing BMI to classify obesity status [18]. With the rapid increase of obesity prevalence worldwide, attempts to develop a simple and low cost method to estimate adiposity more accurately than BMI are being made [170-172].

1.6.2 - The Body Adiposity Index (BAI) and Obesity

Due to significant limitations of BMI to accurately predict adiposity and the impracticality of direct percent body fat measurements from magnetic resonance imaging (MRI) and dual-energy x-ray absorptiometry, a study by Bergman et al. [170] proposed a new equation to calculate adiposity. Utilizing regression analysis the aforementioned study found that height and hip circumference, measured in meters (m) and centimeters (cm) respectively, were sufficient to produce an equation to accurately predict the body fat measured by DXA. The Body Adiposity Index (BAI) equation was derived from the concordance between the body fat percentages of an Mexican-American population. Unlike BMI, the BAI (hip circumference (cm)/[(height (cm)$^{1.5}$)-18]) derived from this investigation did not involve the measurement of body weight. Bergman et al. [170] reported that their BAI equation could more reliably predict body fat than BMI and required no statistical correction for gender or ethnicity [170]. However, the predictive of BAI for %BF remains inconclusive and it remains unclear whether BAI is a better predictor of %BF than BMI [170, 173-179]. Moreover, it is also unclear if BAI will reflect the gender differences of height and hip circumference measurements [180, 181]

along with various dynamic anatomical measurements utilized to evaluate adiposity. Lastly, caution must be exercised when interpreting the association of BAI with %BF as not all studies have employed a high resolution measurement of body fat, such as DXA. A letter to the editor by Barreira et al. [182] was the first published material regarding the validation of the BAI equation on a Caucasian population. They found that the correlation between body fat percentage with BAI was very similar to that with BMI ($r = 0.82$ and $r = 0.83$ respectively) in women. However, the correlation of %BF with BAI was not as similar as BMI ($r = 0.75$ and $r = 0.81$) for men. Barreira et al. concluded that BAI equation was an effective method for predicting %BF for a Caucasian population, although further studies are required to support these findings. Since very few studies had attempted to validate the BAI equation on a Caucasian population our group became interested in doing so in the Caucasian Newfoundland and Labrador population. The purpose of the work described in **Chapter 6** was to validate the predictive power of BAI for %BF, measured by DXA and to investigate the potential influence of adiposity and gender on the accuracy of BAI. Our manuscript was among the first of its kind to attempt to validate the accuracy of the BAI in its prediction of %BF, measured by DXA, among normal-weight, overweight, and obese Caucasian men and women.

1.6.3 - The Association of the Body Adiposity Index With Obesity-Related Cardiometabolic Risk Factors

The association of cardiometabolic risk factors (CRF) with obesity is not well established. The majority of epidemiological studies/reports attempting to evaluate the

association of CRFs with obesity are based upon the assumption that BMI accurately predicts adiposity. However over the past few decades, especially with the advent of new high resolution measurements of adiposity, our laboratory [15-17, 178] and others [14, 15, 167-169, 183-186] have demonstrated that BMI is a very poor predictor of adiposity and obesity status. More importantly, the false-negative classification of BMI defined obesity status reduces the identification of those at higher risk of cardiometabolic diseases with lower body fat content [187]. Therefore, as mentioned in the previous section *" The Body Adiposity Index and Obesity"*, recent focus has been placed on developing more accurate and cost effective methods of assessing adiposity to determine the more precise link between body fat accumulation and its associated disease risk [170, 188, 189]. To date a small number of studies to date have attempted to validate the effectiveness of BAI to predict body fat, and an even smaller number of studies have investigated the association of BAI and BMI with CFRs [190-194] and even fewer studies have included both insulin resistance and heart disease risk factors in their investigation [190, 191]. Despite the current criticisms of the use of BAI equations to predict %BF, an equally important question is; Does BAI more closely represent the association of %BF with obesity-related CFRs than BMI. The current literature demonstrates that [190-192, 195-198], BMI significantly over estimates the association of obesity with CRFs. However, since a number of studies have shown that weight is more associated with CRF than hip circumference independent of sex, it is not surprising that BMI was found to be more strongly associated with CRFs than BAI. A study by Snijder et al. [198] and Freedman et al. [199] both found that BMI and waist circumference were

more strongly correlated than BAI with LDLc, HDLc, TG, glucose, systolic blood pressure and diastolic blood pressure. An investigation from the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) study found that BMI was a better predictor of circulating lipids and glucose than BAI. BMI was more strongly associated with HDLc, TG, glucose, insulin, and HOMA-IR than BAI [192]. Another study reported that BMI was more significantly associated with glucose, systolic blood pressure and diastolic blood pressure [195]. A weight loss study on obese post-menopausal women by Elisha et al. [196] found that the reduction in BAI was not associated with that the changes of CRFs (cholesterol, HDLc, TG, glucose, systolic blood pressure and diastolic blood pressure). None of the above studies evaluated whether BMI or BAI was more closely associated with %BF. Therefore, it would be equally important to evaluate whether the association of BAI or BMI with CRFs was more concordant with that of %BF. The majority of studies seem to indicate that BAI is an inferior predictor of CRFs than BMI. However, to the best of our knowledge no large cross-section study has compared the association of insulin resistance and cardiovascular disease risk factors with BMI and BAI against their associations with a high resolution measurement of body fat while also taking into considering both gender and adiposity. Currently, there is no consensus as to which method, the BAI or BMI can be more closely associated with CFRs. This might be more important in clinical practice. Therefore, the purpose of the manuscript in **Chapter 7** was to determine 1) the association of BAI and BMI with CRFs against the association of DXA %BF with CRFs while taking into consideration both gender and adiposity and 2) whether BAI, or BMI, is more closely associated the

relationship of adiposity (%BF measured by DXA) with cardiovascular disease and type-2 diabetes risk factors. We hypothesize that BMI may be more strongly associated with CFRs, but the association of BAI with CFRs will more accurately reflect the association of CFRs with body fat independent of gender.

1.6.4 - Designing a New Sex Specific Predictive Adiposity Equation

Even though a number of studies have shown that BAI is as good or better a predictor of %BF [173, 175, 178, 189, 194, 197, 200, 201], these studies have also revealed that this association is significantly reduced when attempting to predict adiposity with BAI among men and women separately. The BAI tends to over-estimate, and underestimate DXA %BF among men and women, respectively [174, 189,201]. Additionally, the waist circumference of men had stronger concordance with DXA %BF than that of the hip circumference measurement used in the BAI equation [178, 197]. Recent, studies have also revealed that the predictive power of BAI significantly decreases among underweight and obese individuals [173,174,178,201-204]. As the incidence of obesity and obesity-related diseases continues to increase, the need for an inexpensive and accurate assessment of adiposity has become of great importance to both clinical and epidemiological research. To have an equation that can accurately estimate adiposity for both men and women in the general population would be ideal due to the inherent gender differences. However, considering that hip circumference, the primary measure in the BAI equation, is larger in females than in men [15] it could potentially reflect gender differences to some degree. To date very few sex specific adiposity

equations, which accurately predict high resolution %BF measured by DXA, have been developed. Since the recently BAI does not overcome the weakness of the BMI in predicting adiposity, the purpose of the study described in **Chapter 8** was to develop an equation(s) to more accurately predict body fat percentage among men and women to better define obesity status. An equation such as this would be of critical importance to determine the biomarkers related to obesity development and obesity-related comorbidities.

2

Chapter 2: The Association of Serum Total Peptide YY (PYY) with Obesity and Body Fat Measures in the CODING Study

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2.1 - INTRODUCTION

Peptide YY (PYY), a 36 amino acid appetite suppressing gut hormone, is secreted from the L-cells of the gastrointestinal tract. Low levels of PYY have been reported to be associated with higher BMI and obesity [53]. Circulating PYY increases satiety and subsequently decreases food intake via gut-brain communication [53, 54, 59],inhibits gastrointestinal motility[205]and pancreatic hormone secretion[58, 206].In addition, it hasbeen documented that PYY plays an integral part in maintaining energy homeostasis [27, 28]. However, the involvement of PYY in the development of human obesity is unclear[66, 79, 80],the relationship between circulating PYY and adiposity is controversial[69],and very little data exists from large human population-based studies.

Appetite, a key factor in energy homeostasis, significantly influences the regulation of body weight [51]. Therefore investigating appetite regulating hormones, such as PYY, may provide valuable insight into the underlying mechanisms responsible for the development of obesity. Initial PYY knockout studies revealed that mice developed significant hyperphagia and that the acute administration of PYY could significantly ameliorate this condition [30, 53,61]. This group also proposed that endogenous PYY concentration was lower in obese rodents and humans, inversely associated with obesity-related phenotypes [51], and the administration of PYY could effectively reduce food intake and body weight independent of obesity status [30, 51, 53]. Therefore it was hypothesized that a deficiency in circulating PYY could be a significant contributing factor toward the chronic over consumption of food and subsequent weight

gain. However, many successive PYY knockout [63, 64] studies have failed to reproduce the strong association of PYY with hyperphegia and diet-induced obesity. A considerable number of studies have also been unable to replicate the original results in rodents or obese humans [65, 66, 71] along with any significant association between circulating PYY and adiposity [58, 70, 207].

Considering that very few cross-sectional studies with large sample sizes have been performed considering confounding variables and that the current results in the literature are controversial; there is a necessity to explore the association of circulating PYY with obesity at the population level. Therefore the purpose of our study was to investigate the association of circulating PYY concentration with obesity status and body composition, measured by dual-energy x-ray absorptiometry, in a large population adjusting for major confounding factors. The detailed objectives were to: 1) test if PYY is associated with obesity status by comparing fasting serum PYY between normal-weight (NW), overweight (OW) and obese (OB) men and women; 2) examine the association of PYY with body composition among normal-weight (NW), overweight (OW) and obese (OB) men and women; and 3) determine the influence of age, sex, smoking, medication use and menopausal status on circulating PYY.

2.2 - SUBJECTS & METHODS

Ethics Statement

This study was approved by The Health Research Ethics Authority (HREA) for the Faculty of Medicine, Memorial University of Newfoundland and Labrador, St John's,

Canada. All subjects provided written informed consent.

Subjects

A total of 2094 subjects from theComplex Diseases in the Newfoundland population: Environment and Genetics (CODING) study (Male 523, Female 1571) was used for this investigation[15-17, 208,209].All participants of this current study were from the Canadian province of Newfoundland and Labrador. Eligibility of participants for the CODING study was based upon the following inclusion criteria: 1) > 19 yrs of age; 2) at least a third generation Newfoundlander; and 3) healthy, without any serious metabolic, cardiovascular, or endocrine diseases. The primary method of subject recruitment for the CODING study was the use of posters and handouts. This literature was distributed throughout public facilities (offices, and hospitals) in the city of St. John's, Newfoundland and Labrador. Each individual completed a number of questionnaires to obtain information regarding lifestyle and physical activity. Anthropometric, body composition and biochemical measurements were performed following a 12-h fasting period.

Anthropometric and body composition measurements

Height (cm) and weight (nearest 0.1 kg) measurements were collected and Body Mass Index (BMI) calculated. BMI was defined as weight divided by height squared (kg/m^2). Waist circumference (cm) was measured as the horizontal distance around the abdomen at the level of the umbilicus, and hip circumference (cm) was measured as the

largest circumference between the waist and thighs. Height, waist and hip measurements were recorded to the nearest 0.1 cm. Percent body fat (%BF), percent trunk fat (%TF), percent android(abdominal) fat (%AF) and percent gynoid (lower abdominal-thigh)fat (%GF) were measured, in a supine position, utilizing dual-energy X-ray absorptiometry (DXA, Lunar Prodigy; GE Medical Systems, Madison, WI).The Lunar Prodigy software system determines automatically the regions for the assessment of %TF, %AF, %GF. %TF region is from the top of the shoulders to the top of the iliac crest, while the %AF region is the top of the second lumbar vertebra to the top of the iliac crest and the %GF region extends down iliac crest twice the height of the android area. The current version of the enCORE software for the DXA data presented within this manuscript cannot differentiate visceral from subcutaneous fat. Therefore the %TF, %AF, and %GF regions represent the summation of both subcutaneous and visceral fat relative to theseregions. DXA produces an accurate measurement of adipose tissue within the body with a low margin of error. For this reason, DXA is considered to be one of the most reliable measurements of adiposity and is commonly used as a standard compared to less accurate field methods such as BMI. The enCORE (Ver 12.2, 2008, GE Medical Systems, Madison, WI) software package was used for DXA data acquisition. Quality assurance was performed on the DXA scanner daily and the typical CV during the study period was 1.4%.

Total PYY Measurement

Fasting blood samples were obtained and serum was stored at -80 °C for

subsequent analyses. Serum total PYY concentration was determined via an enzyme-linked immunosorbentassay (Millipore Corporation Pharmaceuticals, Billerica, MA, USA).Due to the large number of participants in this current investigation samples were measured in singlet. However the intraassay (range of 4.8% to 5.4%) and interassay (5.1%) variation from our previous study, [207]and the measurements of PYY in the current study, were performed by the same research assistant. The detection limit of the PYY enzyme-linked immunosorbent assay used was 10 pg/mL for a sample size of 20 µL.

Lifestyle Assessment

All participants completed a self-administered screening questionnaire, which queried covariates including smoking (smoker, non-smoker) and medication (medication user, non-medication user). Female subjects were screened for menopausal status (Pre-menopause, Post-menopause) by questionnaire.

Data Analysis

Data are presented as means ± standard deviation (SD) unless otherwise stated. Participants with PYY concentrations falling outside the range of ± 3SDs (n = 27) were considered outliers and excluded from analyses. The influence(s) of sex (Men vs. Women) environmental factors(smoking vs. non-smoking, medication vs. non-mediation user) and menopause (pre-menopause vs. post-menopause)on PYY concentration were assessed using independent sample t-tests. Pearson correlation analysis was also

performed to assess the association of circulating whole PYY with body composition measurements for the entire cohort along with males and females separately. Obesity status (normal-weight, overweight, and obese) for participants (n = 2094) was determined based on %BF according to the, age and sex specific, criteria recommended by Bray [3].Obesity status was also grouped based on BMI as normal-weight (NW; 18.5–24.9 kg/m^2), overweight (OW; 25.0 – 29.9 kg/m^2) or obese (OB; $\geq$ 30 kg/m^2) according to criteria of the World Health Organization[210]. To further explore the association of PYY with body composition we stratified subjects into tertiles according to waist circumference, hip circumference, waist-hip ratio, BMI, %BF, %TF, %AF and %GF. Participants were also divided into tertiles according to fasting serum PYY concentrations (pg/ml) to examine differences in adiposity measurements. Subsequently, adjusted and unadjusted multiple regression analyses were used to further explore the associations found between body composition measurements and circulating PYY concentration. The effect of age on the relationship between PYY and adiposity was also thoroughly explored. PYY concentrations were compared among normal-weight, overweight, and obese participants, defined by %BF according to the Bray Criteria, from four different age groups: 1) younger than 30 years of age (<30 yrs); 2) 30years of age and greater but less than 40 years of age ($\geq$ 30 yrs - <40 yrs); 3) 40 years of age and greater but less than 50 years of age ($\geq$40 yrs - <50 yrs);and 4) 50 years of age and greater ($\geq$ 50yrs).

Differences in PYY concentration among obesity statuses (NW,OW,OB), body

composition tertiles (low, medium, high),were analyzed by one-way analysis of covariance (One-way ANCOVA) controlling for age, sex, smoking, medication use, and menopause. Multiple regression analysis also included age, sex, smoking, medication use, and menopausal status as potential covariates. R statistical software package version 2.15.2 (R development core team) was used for all analyses. Statistical analyses were two-sided and a P value <0.05 was considered to be statistically significant.

2.3 - RESULTS

PYY concentration and body composition measurements among male and female participants

Body composition characteristics and PYY concentration from both male and female subjects are shown in **Table 2.1**. Independent t-test analysis revealed that PYY, weight, height, BMI, waist circumferenceand waist-hip ratio were significantly greater for men than women. Women were significantly greater than men for age, hip circumference, %BF, %TF, %AF, and %GF. Sex differences for body composition measurements among Newfoundland and Labradoreans have been previously described by us [15-17]. Fasting serum total PYY concentrations were found to be approximately 9%, or 11pg/ml, higher in men than in women (**Table 2.1**).

Association of lifestyle/environmental factors with PYY concentration

The influence of lifestyle and environmental factors such as smoking, medication use, and menopause on circulating PYY concentration were explored. None of these factors had an influence on PYY concentration for men. Female smokers had a 9.7% higher fasting PYY concentration than female non-smokers (120.50 ± 77.6 pg/ml vs 109.87 ± 73.5 pg/ml, $p < 0.04$). PYY concentration was significantly higher for female medication users (114.51 ± 75.5 pg/ml) over non-medications users (106.71 ± 72.4 pg/ml) ($p = 0.04$). With regards to menopausal status, PYY concentration was 10.3% greater for post-menopausal women over pre-menopausal women (117.78 ± 78.3 pg/ml vs106.70 ± 71.5 pg/ml, $p < 0.006$). Age, weight, BMI, waist circumference, hip circumference, %BF, %TF, %AF and %GF were all significantly greater for post-menopausal women compared to pre-menopausal women ($p < 0.004$). Lastly, to investigate the potential compound effect of smoking, medication use, and menopausal status on fasting PYY concentration for women we compared post-menopause smoking medication users (n=53) with pre-menopause non-smoking non-medication users (n=376). The post-menopause smoking medication users group (139.40±93.7 pg/ml) had a 38.6% higher fasting PYY concentration than pre-menopause non-smoking non-medication users (100.60±67.7)($p < 0.005$) independent of age, BMI and %BF.

Pearson correlation analysis of body composition measurements with circulating PYY

Pearson correlation analysis of weight, height, BMI, waist circumference, Hip circumference, waist-to-hip ratio, %BF, %TF, %AF, %GF with circulating PYY are shown in **Table 2.2.** Although no association was found between any of the body

composition measurements with PYY (pg/ml) in men, age (y), weight (kg), height (cm), waist circumference (cm) and the waist-to-hip ratio were significantly positively associated with circulating PYY (pg/ml) in the entire cohort. Additionally, age, waist circumference, hip circumference, %BF, and %TF were significantly positively associated with circulating PYY (pg/ml) in women.

PYY concentration and body composition among normal-weight, overweight and obese for male and females participants

Body composition characteristics and PYY for NW, OW and OB men and women are shown in **Table 2.3**. Fasting total PYY concentration was not significantly different among NW, OW, and OB subjects within the entire cohort or for either sex(**Table 2.3**).Weight, BMI, waist circumference, hip circumference, waist-hip ratio, %BF, %TF, %AF and %GF all significantly increased with the concomitant increase in adiposity status within the entire cohort and for both sexes. There was also no significant difference in circulating PYY when these adiposity groups were classified by BMI according to the WHO criteria (**Figure 2.1**).

PYY concentration and body composition measurements among tertiles (low, medium, and high) according to body composition measurements and PYY concentration

To further explore the potential relationship between PYY and body composition, PYY concentrations were compared among subjects stratified into tertiles according to obesity-related phenotypes (waist circumference, hip circumference, BMI, %BF, %TF,

%AF and %GF). We found no significant difference in PYY concentration among any of the body composition measurement tertiles, or a difference in any of the body composition values among the PYY tertiles, in men (**Table 2.4 & 2.5**). However, PYY concentrations were significantly associated with the increase in waist circumference, %BF, and %TF and these same body composition measurements were significantly associated with the concomitant increase of PYY concentration in women (**Table 2.4 & 2.5**).PYY concentration was also significantly associated with the increase in waist circumference and waist-hip ratio before and after adjustment for the entire cohort. Hip circumference and waist-hip ratio are also positively associated with the increase in circulating PYY before and after adjustment for the entire cohort and women (**Table 2.4 & 2.5**). Lastly, %AF was significantly associated with the increase in circulating PYY concentration in females (**Table 2.4 & 2.5**).

Multiple regression analysis of body composition measurements with circulating PYY

Unadjusted and adjusted linear regression analysis results of %TF, %BF, and waist circumference on circulating PYY are shown in **Table 2.6**. No association was found between %TF (%) or %BF (%) with PYY (pg/ml) in the entire cohort or among men. Although waist circumference (cm)was not associated with circulating PYY in men, it was positively correlated with the entire cohort before and after the adjustment for age, smoking, medication use and menopausal status. Circulating PYY was positively associated with waist circumference (cm), %TF (%), and %BF (%) in women before adjustment for confounding factors. However after the adjustment for age, smoking,

medication use and menopausal status only %TF (%) and waist circumference (cm) remained significantly associated with PYY in women(**Table 2.6**).

Association of age with circulating PYY concentration

Circulating PYY were also analyzed among normal weight, overweight and obese men and women for 4 different age ranges(< 30 yrs,≥30 yrs - <40 yrs,≥40-<50 yrs, and ≥ 50 yrs).No sex differences for PYY concentration were found among normal-weight, overweight and obese for any of the 4 age groups(Data no shown) and circulating total PYY concentration was not significantly different among adiposity groups within any of the 4 age ranges (Data no shown). However, PYY concentration was 15.2 %,17.1% and 11.8% greater among men than women within the < 30 yrs, > 30 - < 40 yrs, and > 40 - < 50 yrsgroups respectively (**Figure 2.2**.). Additionally, the ≥ 50 yrs group from women had 12.2% higher circulating PYY than women in the < 30 yrs group (118.1 ± 77.1 vs. 105.3 ± 63.9)(**Figure 2.2**).

Table 2.1 - Body composition characteristics and PYY concentration[1,4,5]

	Entire Cohort (n = 2094)		Male (n = 523)		Female (n = 1571)		
	Mean	SD	Mean	SD	Mean	SD	P
Age (y) [3]	42.77 ±	12.8	40.00 ±	14.2	43.70 ±	12.2	**< 0.001**
Weight (kg) [2]	73.49 ±	15.3	85.16 ±	14.2	69.60 ±	13.5	**< 0.001**
Height (cm) [2]	165.76 ±	8.5	176.12 ±	6.4	162.29 ±	6.0	**< 0.001**
BMI (kg/m^2) [2]	26.68 ±	4.9	27.50 ±	4.4	26.40 ±	5.0	**< 0.001**
Waist (cm) [2]	91.53 ±	14.2	96.70 ±	13.5	89.80 ±	14.0	**< 0.001**
Hip (cm) [3]	100.72 ±	11.5	99.50 ±	10.0	101.10 ±	11.9	**< 0.003**
Waist-Hip Ratio [2]	0.91 ±	0.1	0.97 ±	0.1	0.89 ±	0.1	**< 0.001**
Body fat (%) [3]	34.79 ±	9.0	25.40 ±	8.0	37.90 ±	7.0	**< 0.001**
Trunk fat (%) [3]	37.00 ±	9.2	30.30 ±	8.8	39.20 ±	8.1	**< 0.001**
Android fat (%) [3]	42.31 ±	10.7	36.54 ±	10.9	44.21 ±	9.9	**< 0.001**
Gynoid fat (%) [3]	41.03 ±	9.6	29.00 ±	7.8	45.03 ±	6.1	**< 0.001**
Peptide YY (pg/ml) [2,4]	113.96 ±	75.2	122.21 ±	77.9	111.21 ±	74.1	**< 0.004**

[1.] All values are means ± SDs. Gender differences were analyzed by a one-way ANCOVA.

[2.] Variable significantly greater in men than women.

[3.] Variable significantly greater in women than men.

[4.] PYY Minimum and Maximum (pg/ml) – Entire Cohort (3.7 – 368.5); Male (7.26 – 364.7); Female (3.67 – 368.5).

[5.] Significance level for one-way ANCOVA (controlling for age) was set to $P \leq 0.05$.

Table 2.2 - Pearson correlation of body composition measurements with circulating PYY[1,2]

	Entire Cohort		Male		Female	
	(n = 2094)		(n = 523)		(n = 1571)	
	r	p	r	p	r	p
Age (y)	0.05	0.031	0.03	NS	0.07	0.009
Weight (kg)	0.05	0.027	-0.03	NS	0.04	NS
Height (cm)	0.05	0.017	0.02	NS	0.01	NS
BMI (kg/m^2)	0.03	NS	-0.03	NS	0.04	NS
Waist (cm)	0.06	0.003	-0.01	NS	0.07	0.003
Hip (cm)	0.04	NS	-0.04	NS	0.07	0.007
Waist-Hip Ratio	0.06	0.008	0.03	NS	0.03	NS
Body fat (%)	-0.01	NS	-0.02	NS	0.05	0.043
Trunk fat (%)	0.01	NS	-0.01	NS	0.06	0.025
Android fat (%)	0.01	NS	0.00	NS	0.05	0.056
Gynoid fat (%)	-0.05	NS	-0.03	NS	0.01	NS

[1.] Pearson correlation of body composition measurements with PYY (pg/ml).

[2.] Statistical significance was set to $p < 0.05$.

Table 2.3 - Body composition measurements and PYY concentration among NW, OW and OB men and women[1,3]

	Entire Cohort			Male			Female		
	Normal	Overweight	Obese	Normal	Overweight	Obese	Normal	Overweight	Obese
	(n = 687)	(n = 628)	(n = 779)	(n = 188)	(n = 132)	(n = 203)	(n = 499)	(n = 496)	(n = 576)
Age (y)	40.0 ± 13.8	44.5 ± 12.1	43.8 ± 12.1	37.2 ± 14.9	43.4 ± 12.9	40.4 ± 13.6	41.1 ± 13.2	44.8 ± 11.9	45.0 ± 11.3
Weight (kg)	64.2 ± 10.3	70.6 ± 11.1	84.0 ± 15.6	76.1 ± 8.7	84.3 ± 9.1	94.3 ± 15.3	59.7 ± 6.5	66.9 ± 8.3	80.4 ± 14.0
Height(cm)	166.7 ± 8.6	164.9 ± 7.9	165.6 ± 8.8	176.7 ± 6.6	175.6 ± 5.5	176.9 ± 6.9	164.0 ± 5.9	162.1 ± 5.7	162.0 ± 6.3
BMI (kg/m^2)	23.0 ± 2.5	25.9 ± 3.0	30.6 ± 4.8	24.4 ± 2.6	27.4 ± 2.7	30.5 ± 4.5	22.5 ± 2.2	25.5 ± 2.9	30.6 ± 4.9
Waist (cm)	81.5 ± 8.7	89.9 ± 10.2	101.7 ± 14.1	87.1 ± 7.9	95.6 ± 7.5	106.3 ± 13.9	79.4 ± 8.1	88.4 ± 10.3	100.0 ± 13.8
Hip (cm)	92.1 ± 7.0	99.4 ± 7.4	109.3 ± 11.2	93.0 ± 7.0	98.5 ± 7.1	106.3 ± 9.6	91.8 ± 7.1	99.6 ± 7.5	110.4 ± 11.5
Waist-Hip Ratio	0.9 ± 0.08	0.9 ± 0.08	0.9 ± 0.09	0.9 ± 0.06	1.0 ± 0.04	1.0 ± 0.09	0.9 ± 0.08	0.9 ± 0.07	0.9 ± 0.07
Body fat (%)	26.5 ± 6.7	35.1 ± 5.5	41.9 ± 6.4	17.4 ± 4.0	25.3 ± 2.1	32.9 ± 3.8	29.9 ± 3.5	37.7 ± 2.1	45.1 ± 3.5
Trunk fat (%)	27.6 ± 6.4	37.7 ± 4.8	44.8 ± 5.7	21.0 ± 5.6	31.1 ± 3.6	38.4 ± 4.0	30.1 ± 4.7	39.4 ± 3.4	47.0 ± 4.3
Android fat (%)	31.7 ± 8.5	43.2 ± 6.1	50.9 ± 6.2	26.0 ± 8.7	37.8 ± 5.8	45.5 ± 5.3	33.9 ± 7.3	44.6 ± 5.3	52.8 ± 5.4
Gynoid fat (%)	34.7 ± 9.0	41.2 ± 7.7	46.5 ± 7.8	22.4 ± 6.4	28.3 ± 4.5	35.6 ± 4.7	39.4 ± 4.2	44.7 ± 3.7	50.3 ± 4.3
Peptide YY (pg/ml)[2]	110.3 ± 73.4	116.4 ± 75.6	115.2 ± 76.4	121.3 ± 79.9	127.0 ± 72.6	119.8 ± 79.4	106.2 ± 70.4	113.5 ± 76.2	113.6 ± 75.3

[1] All values are means ± SDs. Subjects were classified on the basis of percentage body fat as normal-weight (NW), overweight (OW), and obese (OB) according to criteria recommended by Bray.

[2] PYY Minimum and Maximum (pg/ml) – Entire cohort (NW 3.7 - 359.5, OW 3.7 - 364.1, OB 4.5 - 368.5); Male (NW 18.2 - 358.0, OW 3.7 -364.14, 7.3 - 364.7); Female (NW 3.67 -359.5, OW 3.7-364.1, OB 4.5 - 368.5).

[3] Significance level for one-way ANCOVA (controlling for age, gender, smoking, medication use, and menopause) was set to $p \leq 0.05$.

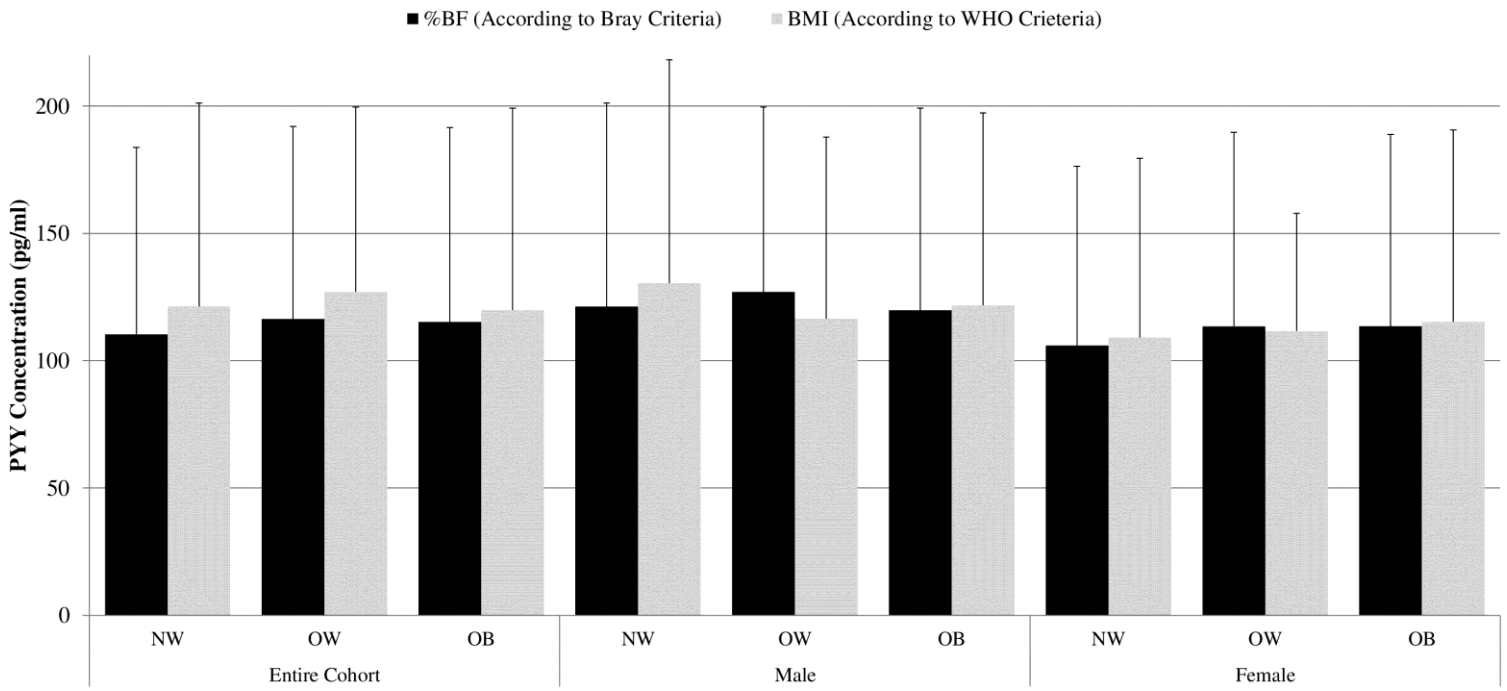

%BF (According to Bray Criteria)
BMI (According to WHO Crieteria)
PYY Concentration (pg/ml)
200
150
100
50
0
NW
OW
OB
NW
OW
OB
NW
OW
OB
Entire Cohort
Male
Female

Table 2.4 - PYY concentrations according to body composition measurements.[1,4,5,6]

	Entire Cohort					Male					Female				
	Low	Medium	High	P	P*	Low	Medium	High	P	P*	Low	Medium	High	P	P*
Waist (cm)[2,3]	109.16 ± 73.4	114.65 ± 75.6	118.23 ± 76.4	**0.023**	**0.024**	122.47 ± 82.2	125.34 ± 78.7	118.80 ± 72.5	NS	NS	106.45 ± 70.2	109.77 ± 73.9	117.64 ± 77.9	**0.01**	**0.02**
Hip (cm)	111.71 ± 74.7	115.15 ± 74.4	115.17 ± 76.5	NS	NS	123.79 ± 83.9	123.11 ± 76.1	119.33 ± 73.2	NS	NS	107.60 ± 71.1	110.61 ± 73.3	115.57 ± 77.7	NS	NS
Waist-Hip Ratio	110.46 ± 74.2	110.81 ± 73.8	120.63 ± 77.1	**0.011**	**0.012**	117.54 ± 75.3	121.87 ± 78.8	127.09 ± 79.5	NS	NS	109.29 ± 75.8	108.75 ± 70.4	115.67 ± 76.0	NS	NS
BMI (kg/m^2)	111.74 ± 75.8	116.85 ± 75.6	113.29 ± 74.1	NS	NS	129.50 ± 85.5	118.13 ± 74.7	118.80 ± 72.6	NS	NS	107.16 ± 71.6	115.64 ± 75.6	110.89 ± 74.9	NS	NS
Body Fat (%)[2,3]	114.41 ± 73.9	112.90 ± 75.8	114.56 ± 75.9	NS	NS	123.72 ± 81.2	125.61 ± 73.9	117.13 ± 78.5	NS	NS	106.22 ± 70.4	112.48 ± 74.7	115.08 ± 76.9	**0.048**	NS
Trunk Fat (%)[2,3]	112.84 ± 75.5	113.07 ± 73.2	115.98 ± 76.9	NS	NS	124.64 ± 81.7	122.75 ± 75.1	119.00 ± 77.0	NS	NS	105.92 ± 71.9	112.20 ± 72.0	115.63 ± 78.1	**0.03**	**0.04**
Android fat (%)	112.76 ± 75.8	114.13 ± 74.6	115.00 ± 75.2	NS	NS	122.80 ± 81.1	122.01 ± 74.6	121.65 ± 78.1	NS	NS	106.87 ± 74.7	111.82 ± 72.0	115.06 ± 75.5	NS	NS
Gynoid fat (%)	119.46 ± 76.5	108.85 ± 72.6	113.53 ± 76.1	NS	NS	123.28 ± 80.8	123.55 ± 74.7	119.61 ± 78.3	NS	NS	108.82 ± 71.2	112.10 ± 75.2	112.85 ± 75.9	NS	NS

[1.] All values are mean ± SDs. Subjects were stratified into tertiles (low, medium and high) based upon body composition measurements.

[2.] Female - Body Fat % (Low 27.7 ± 2.4, Medium 35.7 ± 2.3, High 44.4 ± 3.6); Trunk Fat % (Low 28.3 ± 3.6, Medium 38.0 ± 2.3, High 46.9 ± 3.6); waist circumference (Low 77.2 ± 5.5cm, Medium 89.7 ± 3.3cm, High 107.5 ± 10.1cm)

[3.] Male - Body Fat % (Low 86.2 ± 7.6, Medium 95.8 ± 6.9, High 108.3 ± 13.9); Trunk Fat % (Low 85.9 ± 7.6, Medium 96.2 ± 7.8, High 108.39 ±13.5); waist circumference (Low 83.9± 6.1cm, Medium 95.8 ± 2.3cm, High 110.9 ± 11.3cm)

[4.] P = Differences between sexes and among body composition tertiles were assessed with ANCOVA controlling for age.

[5.] P* = Differences between sexes and among body composition tertiles were assessed with ANCOVA controlling for age, gender, smoking, medication use, and menopause.

[6.] Statistical significance level was set to $p < 0.05$.

Table 2.5 - Body composition according to PYY concentrations.[1,2,3,4,5,6]

	Entire Cohort					Male					Female				
	Low	Medium	High	P	P*	Low	Medium	High	P	P*	Low	Medium	High	P	P*
	(n = 698)	(n = 700)	(n = 696)			(n = 175)	(n = 174)	(n = 174)			(n = 525)	(n = 522)	(n = 524)		
Waist (cm)	90.06 ± 13.3	92.02 ± 14.6	92.52 ± 14.6	**0.0008**	**0.0006**	96.28 ± 14.3	97.55 ± 13.6	96.36 ± 12.5	NS	NS	88.24 ± 12.6	89.94 ± 14.5	91.23 ± 14.8	**0.0003**	**0.0003**
Hip (cm)	100.02 ± 10.9	100.82 ± 11.8	101.31 ± 11.7	**0.03**	**0.02**	99.53 ± 9.5	99.75 ± 10.6	99.31 ± 9.8	NS	NS	100.16 ± 11.3	101.01 ± 12.2	102.17 ± 12.1	**0.004**	**0.007**
Waist-Hip Ratio	0.90 ± 0.1	0.91 ± 0.1	0.91 ± 0.1	**0.007**	**0.006**	0.97 ± 0.1	0.98 ± 0.1	0.97 ± 0.1	NS	NS	0.88 ± 0.1	0.89 ± 0.1	0.89 ± 0.1	**0.021**	**0.015**
BMI (kg/m^2)	26.50 ± 4.5	26.64 ± 5.0	26.91 ± 5.0	NS	NS	27.36 ± 4.3	27.71 ± 4.2	27.38 ± 4.6	NS	NS	26.25 ± 4.6	26.27 ± 5.1	26.73 ± 5.1	NS	NS
Body Fat (%)	34.77 ± 9.0	34.86 ± 8.6	34.75 ± 9.2	NS	NS	24.99 ± 8.0	26.25 ± 7.2	24.91 ± 7.4	NS	NS	37.52 ± 7.1	37.79 ± 7.0	38.46 ± 6.9	**0.023**	NS
Trunk Fat (%)	36.69 ± 9.4	37.23 ± 8.9	37.08 ± 9.3	NS	NS	29.61 ± 9.4	31.42 ± 8.5	29.84 ± 8.6	NS	NS	38.65 ± 8.3	39.19 ± 8.0	39.86 ± 8.0	**0.011**	**0.017**
Android fat (%)	41.76 ± 11.1	42.63 ± 10.5	42.53 ± 10.5	NS	NS	35.64 ± 11.8	37.80 ± 10.3	36.17 ± 10.4	NS	NS	43.40 ± 10.1	44.30 ± 9.9	44.99 ± 9.5	**0.007**	**0.017**
Gynoid fat (%)	41.51 ± 9.4	40.95 ± 9.3	40.63 ± 9.9	NS	NS	29.02 ± 8.5	29.63 ± 7.3	28.36 ± 7.5	NS	NS	45.00 ± 6.0	44.88 ± 6.2	45.24 ± 6.1	NS	NS

[1.] All values are mean ± SDs. Subjects were stratified into tertiles (low, medium and high) based upon PYY Concentration.

[2.] PYY (pg/ml) - Male (Low 50.3 ± 16.9, Medium 103.8 ± 16.7, High 212.9 ± 62.3); Female (Low 43.2 ± 16.9, Medium 93.9 ± 16.2, High 196.6 ± 60.7)

[3.] PYY Minimum and Maximum (pg/ml) - Male (Low 7.3 - 75.3, Medium 75.4- 137.6 , High 138.1 - 364.7); Female (Low 3.7 - 68.6, Medium 68.7 - 125.5, High 125.6 -368.5)

[4.] P = Differences between among PYY Concentration tertiles were assessed with ANCOVA controlling for age.

[5.] P* = Differences between among PYY Concentration tertiles were assessed with ANCOVA controlling for age, gender, smoking, and medication use, and menopause.

[6.] Statistical significance level was set to $p < 0.05$.

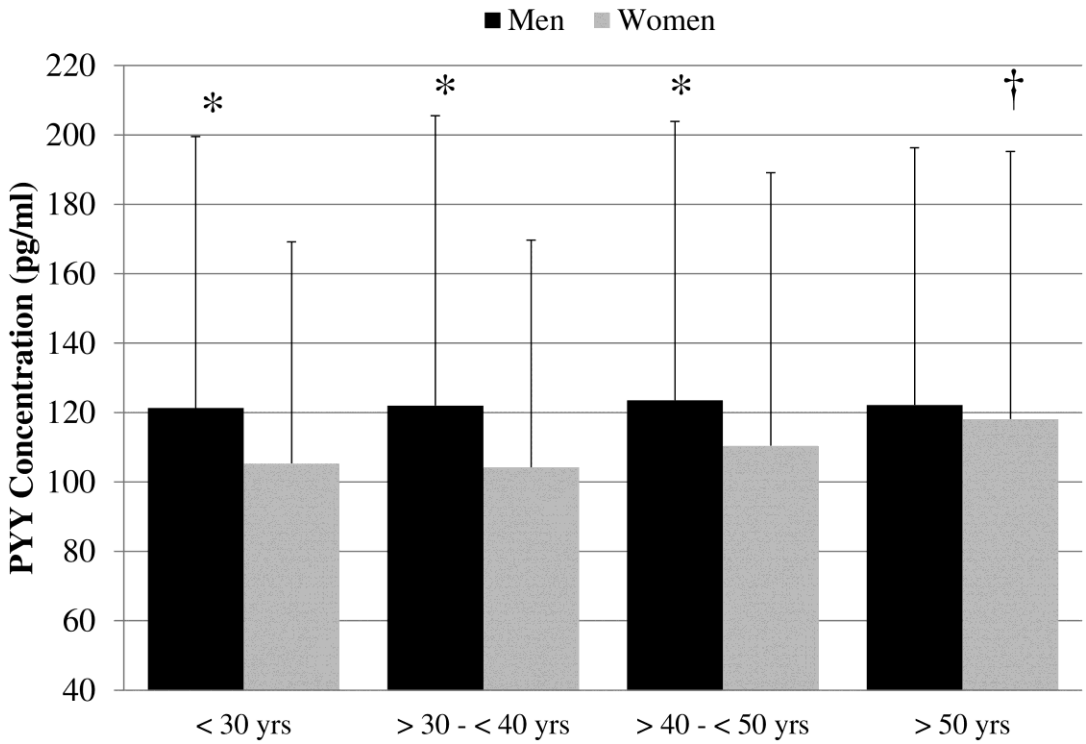
Table 2.6 - Multiple Regression for Body Fat (%), Trunk Fat (%), and Waist Circumference (cm) on PYY Concentration. [1,3]

	Unadjusted[2]		Adjusted [2]	
	β	**p**	**β**	**p**
Body Fat (%)				
Entire Cohort	-0.1083 (0.183)	NS	-0.2984 (0.195)	NS
Male	-0.1984 (0.451)	NS	-0.1368 (0.476)	NS
Female	0.5417 (0.267)	**0.043**	0.2786 (0.285)	NS
Trunk Fat (%)				
Entire Cohort	0.056 (0.179)	NS	-0.1105 (0.194)	NS
Male	-0.1312 (0.386)	NS	-0.1424 (0.421)	NS
Female	0.5170 (0.230)	**0.025**	0.3090 (0.247)	**0.045**
Waist Circumference				
Entire Cohort	0.3414 (0.1154)	**0.003**	0.2982 (0.1208)	**0.02**
Male	-0.0720 (0.253)	NS	-0.0943 (0.2692)	NS
Female	0.3930 (0.133)	**0.003**	0.2826 (0.1437)	**0.049**

[1.] Regression model adjusted for age, gender, smoking, medication use (Menopause was also controlled for in the females).

[2.] β = Unstandardized Beta (standard error).

[3.] Statistical significance was set to $p < 0.05$.


Men
Women
PYY Concentration (pg/ml)
220
200
180
160
140
120
100
80
60
40
*
*
*
†
< 30 yrs
> 30 - < 40 yrs
> 40 - < 50 yrs
> 50 yrs

2.4 - DISCUSSION

One major finding for the present study is that serum PYY is not inversely associated with obesity status defined by BMI or %BF adjusting for age, sex, smoking, medication use, and menopause. Contrary to the current literature we have demonstrated for the first time that fasting serum PYY is positively associated with %BF, %TF, and waist circumference in women. Additionally, circulating PYY was greater among men compared to women, and is affected by age, smoking, medication use and menopausal status in women only.

PYY is an anorexigenic peptide, released from the L-cells of the lower intestine, which acts centrally within the hypothalamus to inhibit the orexigenic activity of the neuropeptide Y (NPY) [30, 52]. The initial investigations involving PYY, conducted in rodents, revealed that a diet-induced obesity state decreased PYY concentrations. Suggesting that the circulating concentration of this appetite suppressing gut hormone is associated with the development of obesity [61, 62]. Studies conducted on a small sample of humans showed that endogenous PYY concentrations were lower in obese subjects and inversely correlated with obesity-related phenotypes [30, 61, 67, 68]. However, other rodent [66] and human based [70, 71, 89, 207] studies have failed to reproduce the strong inverse relationship between circulating PYY and adiposity. Additionally, an extensive review in 2005,exploring the anorexigenic effect of PYY, revealed 84% studies produced among 41 independent research groups were unable to reaffirm the claim proposed by the initial investigations [69].Data from human studies are

limited, inconsistent, contain small sample sizes and have utilized less accurate obesity measures(like BMI)in place of more accurate measures of body fat. Moreover, our laboratory and others have shown that BMI cannot accurately distinguish fat mass from fat-free mass and is not an accurate predictor of adiposity [15, 16]. Therefore the inaccurate measurement of adiposity and the misclassifications of obesity status could be the factor attributing to the contradictory reports concerning the association of PYY with obesity. The first important finding from the present study is that circulating PYY was not significantly different among normal-weight, overweight and obese participants and these findings were consistent whether obesity status was defined either by %BF or BMI. Additionally, a recent study by our laboratory was also unable to detect an association of circulating PYY with obesity status (defined either by BMI or %BF) in a cohort consisting of young men[207].Considering the power of the present study, with over 2000 subjects, our results indicate that circulating PYY concentration is not likely a significant factor affecting obesity status at the population level. However, after extensive analysis we have shown that circulating PYY is positively associated with obesity measures related to body fat in women. PYY concentration was 10.5%, 8.3%, and 9.2% greater among women with the highest waist circumference, %BF and %TF, respectively, compared to women with the lowest aforementioned body fat measures. This finding was also supported by the fact that waist circumference, %BF and %TF were greatest among women with the highest tertile according to PYY concentration and that unadjusted multiple regression analysis revealed a significant positive influence of waist circumference, %BF and %TF. The positive relationship found in our study

between PYY and body fat measures are in direct opposition to the negative association theory. However, among the results of a study [211]designed to investigate various metabolites in insulin resistance, we discovered that PYY concentration was greater among obese subjects compared to normal-weight subjects. Although the primary objective of this study was not investigating PYY and obesity status, their results are consistent with our current findings. The increased level of PYY in circulation is likely a protective response of human body to the obese state. Nevertheless, more studies are needed to verify our findings in other populations.

The potential influence(s) of sex, age, smoking, medication use and menopause on fasting serum PYY concentration were also examined. Studies have reported gender differences in circulating PYY levels. One study found that PYY was higher in men compared to women [72], while others found that PYY levels are higher in women [70].In the present study we discovered that fasting serum PYY was 9% higher in men compared to women. Findings from our current study, along with those of others, suggest that circulating PYY concentration is not significantly affected by age [67, 70,72]. However, when evaluating the influence of age on PYY among females, a positive association was found. Smoking and medication use did not affect PYY concentration in men, but these factors did significantly increase circulating PYY levels in women. Circulating PYY concentration was 9.7 and 7.3% greater among female smokers and medication users over non-smoker and non-medication users, respectively. Currently, there is very little evidence to suggest that smoking and medication use affect circulating

PYY. However some data, from animal models, does propose that nicotine [73-75] and various medications [76-78] can increase circulating PYY. Specifically, rodent studies have shown that nicotine can significantly increase mRNA expression and the production of PYY protein [74] along with the significant attenuation of neuropeptide Y (NPY) mRNA expression and production of NPY protein [73, 75].Therefore, it is probable that the inhibition of appetite, due to smoking, could be partially attributed to the stimulation of PYY and the inhibition of NPY in women. Lastly, post-menopausal women had significantly greater PYY than pre-menopausal women. However, since more than 70% of our post-menopausal women are medication users and that menopause is strongly associated with age, the up regulation of PYY could be an age and drug related interference. Therefore we investigated the potential compound effect of smoking, medication use, and menopausal status on fasting PYY concentration for women. The analysis revealed that post-menopause, smoking, medication users had a 38.6% higher fasting PYY concentration than pre-menopause, non-smoking, non-medication users. This compound effect was unchanged after controlling for age, BMI and %BF. Our study provides strong evidence that there is an obvious sex difference regarding fasting PYY concentration and that age, smoking, medication use and menopausal status all significantly influence circulating PYY concentration in women and not men.

The primary limitation of the present study is that we only investigated total PYY rather than only PYY_{3-36}which has been suggested to be the more significantly active form of PYY. However, total levels of PYY are well correlated with PYY_{3-36}[61] and

both PYY_{3-36} and PYY_{1-36} have been found to decrease gastrointestinal motility [58]. Secondly, due to a significant number of subjects in the current study, singlet measurements of PYY were collected from a single serum sample for each subject. However, due to the fact that the intraassay (range of 4.8% to 5.4%) and interassay (5.1%) variation from our previous study[207] were low, and that the measurement of PYY in the current study was performed by the same research assistant, we are confident that our data is accurate. Moreover, the large sample size further strengthens the reliability. Thirdly, although our findings strongly suggest that PYY is not a significant player in determining obesity status, it must be acknowledged that our samples were collected in a fasted state. Population studies, monitoring the dynamic postprandial response of PYY concentration to various standardized diets, should be performed.

In summary, the association of fasting serum PYY concentration with obesity status and body fat measures was investigated among 2094adultsfrom the Canadian province of Newfoundland and Labrador. To the best of our knowledge this is the largest cross-sectional study to systematically evaluate the relationship between peripheral circulating PYY concentrations with adiposity. Our results indicate that PYY is not significantly associated with obesity status defined by either %BF or BMI. However, circulating PYY is influenced by age, smoking, medication use, and menopausal status in women. Moreover contradictory to previous studies, fasting serum PYY in the present study was significantly associated with waist circumference, %BF, and %TF in women. Although the effect size of the positive association of PYY with adiposity in women is

small, and potentially negligible, it may in fact represent a protective response to extreme weight gain.

3

Chapter 3: Serum PYY in Response to Short-term Overfeeding in Young Men.

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3.1 - INTRODUCTION

It has been documented that gut hormones influence appetite and play an integral part in maintaining energy homeostasis through gut-brain communication [27, 28]. Peptide YY (PYY), a short 36 amino acid protein with structural homology to neuropeptide Y (NPY) and pancreatic peptide (PP), is a gut hormone that increases satiety consequently decreasing food intake [59]. PYY is released from the mucosa in the ileum and colon of the gastrointestinal tract existing in a PYY_{3-36} and PYY_{1-36} form [53, 54]. Both forms are physiologically active decreasing pancreatic secretions and suppressing gastrointestinal motility [58]. PYY secretion increases in response to food consumption and is positively correlated with the calorie content of meals [55-57]. However, at the present time it is not clear whether or not PYY concentration is dependent upon adiposity status or if it plays an important role in the development of obesity.

The majority of PYY studies conducted to date have been performed on rodents. It was initially observed that PYY concentrations were decreased after a diet-induced obese state in mice suggesting that altered PYY secretion is associated with the development of obesity [61, 62]. In some human studies endogenous PYY concentrations were found to be lower in obese subjects and inversely correlated with body mass index (BMI), suggesting that PYY deficiency may contribute to obesity [51, 61, 67, 68]. In addition, the infusion of PYY was found to decrease the appetite of both lean and obese subjects along with a subsequent reduction in food intake [51, 53]. Taken

together, these data suggest that individuals with a lower concentration of PYY, such as overweight/obese individuals, may have a weaker satiety signal subsequently leading to the over consumption of food and weight gain. In contrast to these studies many other studies in rodents [66] and humans with larger sample sizes [70, 71,89] could not replicate the relationship between PYY and adiposity status. The administration of PYY was also found not to have any effect on appetite or weight loss [66, 71]. At the present time data regarding the role PYY plays in determining adiposity status is contradictory. In addition, there is no data concerning the effect of a positive energy balance on PYY and the potential difference among normal weight, overweight and obese subjects.

Our laboratory and others have demonstrated that changes in nutritional status, such as overfeeding, have major effects on adipose tissue metabolism [91, 92], circulating concentrations of adipokines [212] as well as gene expression profiles [90]. A positive energy balance may also influence circulating levels of gut hormones such as PYY and subsequently affect appetite. Although recent studies have suggested that PYY plays an important role in energy homeostasis [58, 89], no study has yet investigated the regulation of PYY after a short-term positive energy challenge. The response of PYY to changes in nutritional status will provide insight regarding the potential role of this gut hormone in the development of obesity as well as other related phenotypes including insulin resistance and serum lipid profiles. The objective of the present study was to investigate the role that PYY plays in the development of obesity by investigating the following: 1) fasting serum PYY levels in normal weight, overweight and obese healthy young men at baseline before overfeeding; 2) the response of PYY to short-term

overfeeding between different adiposity statuses; and 3) the correlations of PYY with phenotypes of insulin resistance, glucose, and lipid metabolism.

3.2 - SUBJECTS & METHODS

Subjects were recruited from an overfeeding study investigating the effects of a positive energy balance on endocrine factors as well as parameters of glucose and lipid metabolism [90-92]. A total of 69 young men were recruited from the city of St. John's (Newfoundland and Labrador, Canada) and the surrounding areas. Inclusion criteria were as follows: 1) male; 2) 19–29 yr of age; 3) at least third-generation Newfoundlander; 4) healthy, without any serious metabolic, cardiovascular, or endocrine disease; 5) not receiving medication for lipid metabolism; 6) reported stable weight (±2.5 kg) in the previous 6 months and 7) subjects abstained from any alcoholic or additional calorie-containing beverage consumption during the study period. No study participants took any drugs/medications throughout the duration of the study. Initial data collection for this study began October 2003. This study was approved by The Human Investigations Committee for the Faculty of Medicine, Memorial University of Newfoundland and Labrador, St John's, Canada. All subjects provided written informed consent.

Serum measurements

Fasting blood samples were obtained from all subjects before and after the completion of the overfeeding intervention. Serum was stored at -80 °C for subsequent

analyses. Whole PYY (Millipore Corporation Pharmaceuticals, Billerica, MA, USA) concentrations were measured in duplicate utilizing enzyme-linked immunosorbentassays. The intra-assay variation ranged from 4.8 – 5.4% and the inter-assay variation was 5.1%. The detection limit of the PYY ELISA kits used was 10pg/mL when using a 20 μL sample size. Serum insulin concentrations were measured with the use of an immunoassay analyzer (Immulite; DPC, Los Angeles, CA). The homeostasis model assessment (HOMA) was used as a measure of insulin resistance [HOMA-IR= insulin (μU/mL) × glucose (mmol/L)/22.5)] and β cell function [HOMA-β = 20 × insulin (μU/mL)/(glucose - 3.5)] [213]. Serum concentrations of glucose, triacylglycerols (TG), high-density lipoprotein (HDL) cholesterol and total cholesterol were measured by an Lx20 analyzer (Beckman Coulter Inc, Fullerton, CA). Low-density lipoprotein (LDL) cholesterol was calculated with the following formula: Total cholesterol – HDL cholesterol – (TG/2.2). The LDL cholesterol calculation is reliable in the absence of severe hyperlipidemia.

Measurement of body composition

Height and weight measurements were collected and BMI calculated. BMI was defined as weight divided by height squared (kg/m^2). Percent body fat (%BF), percent trunk fat (%TF), and percent android fat (%AF), were measured utilizing dual-energy X-ray absorptiometry (DXA, Lunar Prodigy; GE Medical Systems, Madison, WI). Measurements were performed on subjects in a supine position, after the removal of all metal accessories, as previously described by us [91, 208]. Version 12.2 of the encore

(enCORE,Ver 12.2, 2008, GE Medical Systems, Madison, WI) software package was used for DXA analysis. Subjects fasted for 12 hours before data was collected. All measurements were collected on the first day of data collection and one day after the 7-day overfeeding intervention.

Overfeeding protocol

A 7-day overfeeding protocol was chosen for this study to ensure that the intervention would induce metabolic changes. Participants consumed 70% more calories than their normal energy requirements (hypercaloric), which consisted of 15% protein, 35% fat, and 50% carbohydrates to mimic the common daily diet in North America. Baseline energy requirements were determined from three 24-hr diet recalls and a 30-d dietary inventory. The average caloric intake per day was as follows: energy (baseline: 2969 kcal; overfeeding: 5471 kcal), protein (baseline: 106 g; overfeeding: 178 g), carbohydrates (baseline: 394 g; overfeeding: 713 g), fiber (baseline: 19 g; overfeeding: 33 g), total fat (baseline: 105 g; overfeeding: 221 g), saturated fat (baseline: 38 g; overfeeding: 71 g), and cholesterol (baseline: 304 mg; overfeeding: 735 mg). Subjects were offered meals at 0900, 1200, and 1700 every day, and energy values and macronutrient content of the food were measured by using FOOD PROCESSOR SQL software (version 9.5.0.0; ESHA Research, Salem, OR). The actical physical activity monitor (Mini Mitter Co, Inc, Bend, OR) was used to determine the total energy expenditure. Physical activity levels were controlled below 15% between the baseline and overfeeding period. Full details of our overfeeding protocol have been previously

described and all of the participants in this study were members of these studies [90-92]. On average, subjects gained 2.4 ± 1.3 kg body weight, of which 43.2 ± 31.6% (0.830 kg ± 0.542) was body fat.

Statistical analysis

Data are presented as means ± SDs unless otherwise stated. Before any statistical analysis was performed, data that were not normally distributed were logarithmically transformed (TG, insulin, HOMA-IR and HOMA-β) to approximate normal distribution. Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (≥ 26%) according to criteria recommended by Bray[3]. Statistical analyses were also performed according to BMI as normal weight (≤ 24.9 kg/m^2), overweight (25.0 – 29.9 kg/m^2) or obese (≥ 30 kg/m^2) according to criteria of the World Health Organization[210]. To further explore the relationship between PYY and body composition, participants were divided into tertiles according to baseline fasting serum PYY concentrations (pg/ml) as follows: Low (Bottom 33.3%), Medium (middle 33.3%) and High (top 33.3%) PYY concentration. The range of serum PYY for the Low, Medium and High groups were 23.85-90.73 pg/ml, 97.69-131.36 pg/ml, and 133.52-347.52 pg/ml respectively.

Differences in variables between the three adiposity groups in response to overfeeding were analyzed by using a mixed model repeated two-way analysis of variance and baseline values between the three adiposity groups were analyzed using a

one-way analysis of variance. The Bonferroni post-hoc test was run after the one-way ANOVA and the two-way ANOVA displaying a significant overfeeding by adiposity interaction ($p < 0.05$). Within group analysis on the response to overfeeding was performed with a student paired t-test performed on variables that displayed a significant overfeeding by adiposity interaction effect. Pearson's correlation analyses were performed to screen for potential factors related to fasting PYY concentrations followed by partial correlation analyses after controlling for age. Bonferroni testing was applied to correct for multiple comparisons. Three correlation analyses were performed as follows: 1) PYY at baseline was compared with all variables at baseline; 2) PYY at baseline was compared with changes in all variables in response to overfeeding to investigate whether baseline PYY could predict the changes in related markers; and 3) Changes in PYY were compared with changes in all variables in response to overfeeding. SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA) was used for all analyses. Statistical analyses were 2-sided and $p < 0.05$ was considered to be statistically significant.

3.3 - RESULTS

Comparison of characteristics at baseline and in response to short-term overfeeding

Physical and biochemical characteristics of subjects at baseline are shown in **Table 3.1**.The body composition, glucose and lipid metabolism differences found between normal weight, overweight and obese young health males have been previously described by us [90-92]. Fasting serum PYY concentrations at baseline for normal weight

(121.47 ± 60.8 pg/ml), overweight (109.90 ± 53.8 pg/ml) and obese (118.93 ± 56.54 pg/ml) subjects were not significantly different from one another. PYY concentrations were also analyzed according to BMI criteria where no significant differences were found (data not shown).

Changes in body composition and phenotypes of glucose metabolism and lipids in response to the 7-day overfeeding are also described in **Table 3.1**.Our 7-day positive energy challenge for normal weight, overweight and obese young healthy males significantly increased body composition, serum lipids, insulin resistance and β cell function. These findings have been previous described by us as well [90-92]. The only significant overfeeding x adiposity status interactions found from our analysis were for %BF and %TF, indicating that normal weight subjects experienced a greater significant increase in body fat percentage than overweight and obese (**Table 3.1**). Regarding whole PYY, there was no significant difference in concentration between normal weight, overweight and obese young men after overfeeding ($p = 0.758$). However, the main effect for overfeeding did reach significance demonstrating that PYY concentration was significantly increased due to the 7-day positive energy challenge ($p = 0.013$).

Correlations of PYY with phenotypes of glucose and lipid metabolism

Correlations between baseline PYY and baseline phenotypes were assessed (**Table 3.2**). In the entire cohort at baseline, PYY was negatively correlated with BMI. However, when these analyses were repeated according to adiposity status the correlation

was only found in normal weight and overweight subjects. PYY was not significantly correlated with %BF for the entire cohort, but was found to be negatively correlated with %BF within the normal weight subjects. Baseline PYY was negatively correlated with baseline %AF within the normal weight group. Correlations between baseline PYY and the changes in phenotypes were also assessed to determine if PYY could act as a predictor of these parameters under conditions of an energy surplus (**Table 3.3**).Three significant negative correlations were found within the normal weight group between baseline PYY and the changes in total cholesterol, LDL-cholesterol and HDL-cholesterol due to overfeeding. Also, baseline PYY was negatively correlated with the changes in glucose for overweight subjects. Correlations between changes in baseline PYY and the changes in phenotypes were also assessed (**Table 3.4**). There were only two positive partial correlations found between the changes in PYY with the changes in phenotype markers due to overfeeding which were HDL-cholesterol and glucose for normal weight subjects (**Table 3.4**).

Comparison of body composition measures in low, medium and high baseline fasting serum PYY concentrations.

Lastly, we wanted to investigate the relationship between Low, Medium, and High baseline fasting serum PYY concentrations and markers of body composition. The between group comparison demonstrated that there were no significant differences in the any of the body composition variables at baseline nor after the overfeeding protocol (body weight, BMI, %BF, %TF, %AF). %BF at baseline stratified in the Low, Medium,

and High PYY concentrations was 24.54 ± 8.2%, 22.6 ± 8.9% and 22.13 ± 8.5% respectively and were not significantly different from one another ($p = 0.606$). BMI at baseline stratified in the Low, Medium, and High PYY concentrations were 26.66 ± 4.8 kg/m^2, 24.80 ± 4.7 kg/m^2 and 25.14 ± 4.4 kg/m^2 respectively and were also not significantly different from one another ($p = 0.469$). The two-way ANOVA again revealed that the 7-day overfeeding effectively increased whole PYY concentrations ($p = 0.002$) and that there was a significant overfeeding by PYY concentration group interaction ($p < 0.001$). Sub-group analysis revealed that PYY significantly increased within the Low baseline PYY group from 61.87 ± 19.43 pg/ml to 103.51 ± 37.01 pg/ml ($p \leq 0.05$) and that the Medium (from 114.37 ± 10.30 pg/ml to 115.18 ± 43.37 pg/ml) and High (from 178.03 ± 50.14 pg/ml to 194.43 ± 85.72 pg/ml) baseline PYY concentration groups remained unchanged after overfeeding.

Table 3.1 - Physical and biochemical characteristics of subjects at baseline and in response to 7-days of overfeeding[1]

	Normal-Weight[2] (n = 23 - 27)		Overweight[2] (n = 14)		Obese[2] (n = 23 - 28)	
	Before	**After**	**Before**	**After**	**Before**	**After**
Age (y)	23.72 ± 3.6	NA	21.97 ± 3.1	NA	23.24 ± 2.6	NA
Height (cm)	179.51 ± 6.4	NA	179.38 ± 4.6	NA	179.07 ± 6.8	NA
Weight (kg) [4,6,7]	72.39 ± 9.2	74.53 ± 9.6	77.81 ± 4.2	79.39 ± 4.3	93.01 ± 15.6	95.65 ± 16.0
BMI (kg/m^2) [4,6,7]	22.55 ± 2.6	23.23 ± 2.8	24.13 ± 1.3	24.63 ± 1.5	29.10 ± 4.9	29.93 ± 5.0
Percent Body Fat (%) [3,7,8]	14.63 ± 3.3	15.38 ± 3.4 [9]	22.54 ± 0.8	22.82 ± 1.1 [9]	31.51 ± 5.0	31.26 ± 4.7
Percent Trunk Fat (%) [3,7,8]	16.55 ± 3.6	17.52 ± 3.8 [9]	25.39 ± 1.8	25.79 ± 2.2 [9]	35.22 ± 5.4	34.89 ± 5.1
Percent Android Fat (%) [3,7,6]	19.01 ± 4.4	19.86 ± 4.9	28.84 ± 2.6	29.45 ± 2.7	40.47 ± 7.1	41.06 ± 6.7
Total Cholesterol (mmol/L)	4.39 ± 0.9	4.67 ± 0.8	4.63 ± 0.9	4.72 ± 1.1	4.56 ± 0.7	4.79 ± 0.8
HDL - Cholesterol (mmol/L) [4,6,7]	1.38 ± 0.3	1.47 ± 0.3	1.38 ± 0.3	1.43 ± 0.3	1.19 ± 0.2	1.31 ± 0.3
LDL - Cholesterol (mmol/L)	2.61 ± 0.7	2.67 ± 0.7	2.82 ± 0.7	2.83 ± 0.9	2.79 ± 0.7	2.79 ± 0.6
Triacylglycerol (mmol/L) [5,6,7]	0.90 ± 0.3	1.16 ± 0.8	0.92 ± 0.3	1.00 ± 0.5	1.37 ± 0.7	1.58 ± 0.9
Glucose (mmol/L)	4.97 ± 0.4	5.03 ± 0.5	5.03 ± 0.4	5.09 ± 0.6	5.28 ± 0.7	5.17 ± 0.5
Insulin (pmol/L) [4,6,7]	42.86 ± 23.5	62.98 ± 23.8	69.51 ± 69.16	88.85 ± 86.3	96.99 ± 91.8	111.97 ± 77.1
HOMA-IR [4,6,7]	1.39 ± 0.8	2.09 ± 0.9	2.35 ± 2.68	2.95 ± 2.9	3.51 ± 3.8	3.85 ± 2.9
HOMA-β [4,6,7]	83.32 ± 38.6	125.63 ± 49.4	120.21 ± 74.09	175.90 ± 163.6	147.49 ± 90.62	189.67 ± 103.4
Peptide YY (pg/mL) [6]	121.47 ± 60.8	148.44 ± 93.37	109.90 ± 53.8	119.49 ± 38.9	118.93 ± 56.54	136.47 ± 58.27

[1] All values are means ± SDs. PYY, Peptide YY; HOMA - IR, Homeostasis model assessment of insulin resistance; HOMA - β of β cell function; NA, not applicable. Adiposity status and response to overfeeding analyzed by 2 - factor mix model ANOVA (SPSS, version 17.0 Chicago, IL, USA) for repeated measures.

[2] Subjects were classified on the basis of %BF as either normal weight (8 - 20.9%), overweight (21 - 25.9%) or obese (> 26%) according to criteria recommended by Bray.

[3] Significant difference between normal weight, overweight and obese subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[4] Significant difference between normal weight vs obese subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[5] Significant difference between obese vs normal weight and obese vs overweight subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[6] Significant difference due to overfeeding (2 - Way mixed model ANOVA, $P < 0.05$).

[7] Significant difference due to adiposity status (2 - Way mixed model ANOVA, $P < 0.05$).

[8] Significant overfeeding by adiposity status interaction (2 - Way mixed model ANOVA, followed by a Bonferroni corrected test when significant, $P < 0.05$).

[9] Significant difference within group (paired *t*-test, $P < 0.05$).

Table 3.2 - Partial correlations of baseline variables related to baseline fasting serum peptide YY (PYY), after control for age[1]

	All Subjects (n = 60 - 69)		Normal-weight[2] (n = 23 - 27)		Overweight[2] (n = 14)		Obese[2] (n = 23 - 28)	
	r	p	r	p	r	p	r	p
Weight	-0.086	NS	-0.303	NS	-0.314	NS	0.121	NS
BMI	-0.247	0.044[3]	-0.484	0.012[3]	-0.622	0.023[3]	-0.148	NS
Percent Body Fat	-0.151	NS	-0.391	0.048[3]	-0.308	NS	-0.271	NS
Percent Trunk Fat	-0.114	NS	-0.349	NS	-0.254	NS	-0.124	NS
Percent Android Fat	-0.147	NS	-0.431	0.028[3]	-0.307	NS	-0.158	NS
Total Cholesterol	-0.058	NS	0.228	NS	-0.007	NS	-0.317	NS
HDL-Cholesterol	0.056	NS	0.192	NS	-0.057	NS	-0.116	NS
LDL-Cholesterol	-0.148	NS	0.108	NS	-0.014	NS	-0.381	NS
Triacylglycerols	0.176	NS	0.199	NS	0.300	NS	0.180	NS
Glucose	-0.135	NS	-0.076	NS	0.261	NS	-0.272	NS
Insulin	0.034	NS	-0.125	NS	0.413	NS	-0.061	NS
HOMA-IR	-0.027	NS	-0.134	NS	0.434	NS	-0.151	NS
HOMA-Beta	0.061	NS	-0.125	NS	0.481	NS	0.046	NS

[1] PYY, Peptide YY; HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for potential factors related to fasting PYY.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

Table 3.3 - Partial correlations of changes in variables due to short-term overfeeding related to baseline fasting serum PYY, after control for age[1]

	All Subjects (n = 60 - 69)		Normal-weight[2] (n = 23 - 27)		Overweight[2] (n = 14)		Obese[2] (n = 23 - 28)	
	r	p	r	p	r	p	r	p
Weight	0.056	NS	-0.077	NS	-0.004	NS	0.174	NS
BMI	0.057	NS	-0.078	NS	0.000	NS	0.176	NS
Percent Body Fat	-0.067	NS	-0.114	NS	-0.041	NS	-0.115	NS
Percent Trunk Fat	-0.084	NS	-0.104	NS	0.152	NS	-0.210	NS
Percent Android Fat	-0.093	NS	-0.092	NS	-0.561	NS	0.088	NS
Total Cholesterol	-0.090	NS	-0.490	0.011[3]	0.004	NS	0.225	NS
HDL-Cholesterol	-0.142	NS	-0.534	0.005[3]	0.122	NS	0.103	NS
LDL-Cholesterol	-0.083	NS	-0.461	0.018[3]	0.015	NS	0.190	NS
Triacylglycerols	0.088	NS	0.027	NS	0.169	NS	0.203	NS
Glucose	-0.185	NS	-0.318	NS	-0.601	0.039[3]	0.084	NS
Insulin	-0.118	NS	-0.149	NS	-0.350	NS	0.067	NS
HOMA-IR	-0.165	NS	-0.217	NS	0.252	NS	-0.206	NS
HOMA-Beta	-0.010	NS	0.052	NS	0.109	NS	-0.022	NS

[1] PYY, Peptide YY; HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for the potential changes in factors due to overfeeding related to fasting PYY.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

Table 3.4- Partial correlations of changes in variables due to short-term overfeeding related to changes in fasting serum PYY, after control for age[1]

	All Subjects (n = 60 - 69)		Normal-weight[2] (n = 23 - 27)		Overweight[2] (n = 14)		Obese[2] (n = 23 - 28)	
	r	p	r	p	r	p	r	p
Weight	0.019	NS	0.187	NS	-0.006	NS	-0.223	NS
BMI	0.018	NS	0.186	NS	-0.009	NS	-0.225	NS
Percent Body Fat	0.141	NS	0.187	NS	0.372	NS	0.148	NS
Percent Trunk Fat	0.155	NS	0.199	NS	0.318	NS	0.159	NS
Percent Android Fat	0.062	NS	0.164	NS	0.187	NS	-0.178	NS
Total Cholesterol	0.094	NS	0.269	NS	0.110	NS	-0.056	NS
HDL-Cholesterol	0.204	NS	0.507	0.008[3]	0.527	NS	-0.069	NS
LDL-Cholesterol	0.003	NS	0.059	NS	-0.051	NS	-0.018	NS
Triacylglycerols	-0.080	NS	0.236	NS	0.023	NS	-0.362	NS
Glucose	0.101	NS	0.420	0.033[3]	0.013	NS	-0.080	NS
Insulin	0.068	NS	0.057	NS	0.240	NS	0.019	NS
HOMA-IR	0.069	NS	0.062	NS	0.135	NS	0.221	NS
HOMA-Beta	0.036	NS	-0.225	NS	0.157	NS	0.267	NS

[1] PYY, Peptide YY; HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for potential changes in factors due to overfeeding related to the potential changes in PYY due to overfeeding.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

3.4 - DISCUSSION

The gastrointestinal tract is the largest endocrine organ inthe human body and secretes several appetite regulating hormones including PYY. The brainstem and hypothalamus receive neural and hormonal signals through a gut-brain communication pathway subsequently regulating food intake and energy homeostasis [214, 215]. Although PYY is thought to be involved in the development of obesity [30], controversy exists regarding the definitive role that PYY plays in this regard [58, 66, 71]. Therefore, we sought to clarify whether fasting serum PYY varied before and after a positive energy challenge among normal weight, overweight and obese young men.

Data from animal experiments indicate that endogenous PYY acts on the arcuate nuclei through the gut-hypothalamic pathway blocking various orexigenic effects which results in increased satiety and decreased gastric emptying [30, 53, 60]. It has been suggested that fasting serum PYY concentrations are lower in obese rodents as well as humans and that PYY infusion effectively reduces food intake and body weight [51, 53]. However, many other studies have failed to reproduce these findings [66, 71]. In our study, we found no significant differences in fasting serum PYY concentrations between normal weight, overweight or obese men classified by %BF (DXA) or BMI. Furthermore, there were no significant differences between body composition measurements according to DXA or BMI when our subjects were stratified into Low, Medium and High fasting serum PYY concentrations. Therefore, our data do not support the hypothesis that adiposity status, indexed by %BF or BMI, determines basal PYY concentrations. Interestingly, we did observe an inverse correlation between circulating

PYY and markers of adiposity (%BF and BMI) among normal weight subjects. Although the reason for this is unclear, it appears that PYY may have a weak effect on adiposity among normal weight men. Nonetheless, without a number of large population-based studies on fasting serum levels of human PYY, strong conclusions regarding the role PYY plays in determining adiposity status and the development of obesity will remain controversial.

The most important finding from the current study is that the concentration of fasting PYY significantly increased in all subjects after a 7-day overfeeding challenge. In addition, the amount of PYY significantly increased in the low PYY concentration group and not in the medium and high concentration group suggesting a possible dose effect. This finding is difficult to interpret without further study. It was first suspected that the majority of the low baseline concentration PYY subjects experiencing an increase in PYY, from the positive energy challenge, would have been from the normal weight group. However, due to only 9 out of the 23 low PYY concentration subjects being of normal weight we doubt that this possible dose effect would be dependent upon adiposity status. In addition, having divided our subjects into groups according to a baseline measurement it cannot be ignored that the regression to the mean may partially explain both the increase in PYY for the low PYY concentration group and the increase in percent body fat for the normal weight group after our intervention. Previous studies investigating the role of PYY in maintaining energy homeostasis have examined the response of this gut hormone to a negative energy balance [58, 89]. Specifically, a study by Essah et al [89] on 30 obese adults demonstrated that after an 8-week low-fat and low-

carbohydrate hypocaloric diet there was a 9% reduction in body weight and a subsequent 10% reduction in circulating PYY levels [89]. Although the study provides valuable insight into the regulation of PYY by a negative energy challenge, obesity is generally understood to be the result of a chronic energy surplus [86] triggering many complex hormonal changes [87, 88]. The current overfeeding study therefore provides a means in which the biochemical changes in circulating PYY that would be evident with extended overeating, such as in the development of obesity, could be examined. The significant increase in PYY under a positive energy challenge in our study could be physiologically meaningful and act as a protective mechanism to counteract the hypercaloric diet. However, the increase in PYY due to overfeeding is not dependent upon adiposity status which suggests hope that PYY could potentially be helpful to overweight and obese individuals. Our results, combined with data from previous negative energy balance studies, suggest that PYY is involved in maintaining energy homeostasis however the level of contribution is yet to be determined.

Aside from investigating the role PYY plays in body composition and energy homeostasis, we were also interested in investigating its association with parameters of glucose and lipid metabolism. Interestingly, baseline PYY was found to be negatively correlated with the increase in cholesterol measurements (Total, LDL and HDL) in response to overfeeding in normal weight subjects. This finding would suggest that individuals with lower baseline PYY concentrations experience a greater increase in serum lipids after overfeeding. Although the parallel response between PYY and serum

lipids may be beneficial when under a positive energy balance, a causal relationship between these variables cannot be inferred. More studies are warranted to further understand the possible physiological significance between fasting PYY and serum lipids under a positive energy challenge.

The primary limitation of the present study is that we only studied young males (19-29 yr), which limits the application of our conclusions to other groups. Larger population studies investigating the effects of overfeeding with a wider age distribution including females is warranted to determine the physiological role PYY plays in the modification of appetite and the development of obesity. Furthermore, we only investigated whole human PYY rather than PYY_{3-36} which has been suggested to be the more significantly active form of PYY. However, whole PYY levels are well positively correlated with PYY_{3-36}[61] and both PYY_{3-36} and PYY_{1-36} have been found to decrease gastrointestinal motility [58].

In summary, this is the first study of its kind to explore the response of fasting PYY to a short-term positive energy challenge. We measured fasting serum whole PYY in 69 young men before and after a 7-day positive energy challenge. Circulating PYY was similar among normal weight, overweight and obese young men at baseline before overfeeding. We did, however, observe significant negative correlations with both BMI and %BF in normal weight subjects only. Furthermore, PYY was significantly increased in response to the positive energy challenge in the entire cohort. It would appear that

PYY acts as protective mechanism against the development of obesity. The body seems to mount a counter regulatory reaction to prevent further weight gain in normal weight, overweight and obese subjects. Therefore, PYY represents a likely target for further downstream studies related to the development of obesity.

4

Chapter 4: Short-term Overfeeding Increases Circulating Adiponectin Independent of Obesity Status

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4.1 - INTRODUCTION

The accumulation of white adipose tissue (WAT) is the product of a chronic positive energy balance in an obesogenic environment [91]. Numerous disorders such as insulin resistance, type 2 diabetes (T2D) and cardiovascular disease have shown a strong association with the accumulation of subcutaneous and visceral fat mass [216, 217].Once regarded as a site for energy storage, recent research has shown that WAT secretes a large number of physiologically active proteins [113-115] thus playing an integral role in human endocrinology and energy homeostasis [111, 112, 218]. Adiponectin, for example, is predominantly secreted into circulation from adipose tissue, which increases insulin sensitivity resulting in the effective disposal of glucose from circulation [116-118]. Accumulating evidence suggests that adiponectin is strongly associated with glucose and lipid metabolism, although adiponectins role in the development of obesity and insulin resistance remains unclear.

Considering that adiponectin has been recognized as a significant insulin sensitizer it would be consistent to anticipate that circulating adiponectin levels would be considerable in the presence of an insulin resistant state such as obesity. However, the puzzling actuality suggests that adiponectin is inversely associated with obesity [119-124] and insulin resistance [122, 125-127]. Consequently if adiponectin is an anti-obesigenic and anti-diabetic protein, expected to increase in circulation, it is perplexing that adiponectin levels are attenuated in both obese and diabetic populations. Nevertheless there is significant evidence that adiponectin does indeed play a beneficial role in both lipid and glucose metabolism [126, 127, 134,135]. Rodent studies have

shown that adiponectin gene knockout mice severely attenuate glucose disposal and [126, 134] the administration of adiponectin can effectively ameliorate insulin resistance [127, 135]. These findings demonstrate that adiponectin can protect or even reverse obesity related disease states. However, the paradoxical inverse relationship of adiponectin with adiposity remains unclear.

Our laboratory and others have demonstrated that a short-term positive energy challenge affects glucose and lipid metabolism, which results in significant body weight gain and insulin resistance [84 , 91, 92, 207, 212]. Moreover, changes in nutritional status have also shown to significantly affect circulating adipokines[84, 91,92]. For this reason we propose that investigating the response of adiponectin, to adiposity development, will provide insight into the potential role that adiponectin may play in the development of obesity and diabetes. The objective of our present investigation is to address the following as yet unanswered question: Does circulating adiponectin increase in response to a short-term positive energy challenge and is this potential response obesity status dependent. To our knowledge this is the first study of its kind to investigate the effect of a 7-day positive challenge (70% above normal energy requirements) on circulating adiponectin among normal-weight, overweight and obese individuals.

4.2 - SUBJECTS & METHODS

Ethics Statement

This study was approved by The Health Research Ethics Authority (HREA) for the Faculty of Medicine, Memorial University of Newfoundland and Labrador, St John's, Canada. All subjects provided written informed consent. Initial data collection for this study began October 2003.

Subjects

Sixty-four young men, recruited from the city of St. John's (Newfoundland and Labrador, Canada) and surrounding areas, participated in the current positive energy challenge (PEC) study. The study inclusion criteria were as follows: 1) male; 2) non-smoker, 3) 19–29 yr of age; 4) at least third-generation Newfoundlander; 5) healthy young men, without any serious metabolic, cardiovascular, or endocrine disease; 6) not receiving medication for lipid metabolism; 7) reported stable weight (±2.5 kg) in the previous 6 months and 8) subjects abstained from any alcoholic or additional calorie-containing beverage consumption during the study period. Participants refrained from taking any drugs/medications throughout the duration of the study. All physical and biochemical measurements were collected, after a 12 hour fast, on the morning of the first day of overfeeding and one day after the 7^{th} and final day of overfeeding. Study participants were free living throughout the experiment and were not restricted to an experimental environment, such as a hospital.

Serum measurements

Fasting blood samples were obtained from all subjects before and after the completion of the overfeeding intervention. Serum was stored at -80 °C for subsequent

analyses. The majority of biochemical markers [219] and adiponectin [220] remain stable under these storing conditions. Adiponectin (Phoenix Pharmaceuticals, Belmont, California) concentrations were measured in duplicate utilizing enzyme-linked immunosorbentassays. The intra-assay variation ranged from 4.8 – 5.4% and the inter-assay variation was 5.1%. The detection limit of the adiponectin ELISA kits used was 0. 15ng/ml when using 100 μL diluted samples. Serum insulin concentrations were measured with the use of an immunoassay analyzer (Immulite; DPC, Los Angeles, CA). The homeostasis model assessment (HOMA) was used as a measure of insulin resistance and beta cell function [213].

$$HOMA-IR = ((Fasting\_Insulin[\mu U/ml] \times Fasting\_Glucose[mmol/L])/22.5)$$

$$HOMA-\beta = ((20 \times Fasting\_Insulin[\mu U/ml]/(Fasting\_Glucose[mmol/L]-3.5))$$

Serum concentrations of glucose, triacylglycerols (TG), high-density lipoprotein (HDLc) cholesterol and total cholesterol were measured by an Lx20 analyzer (Beckman Coulter Inc, Fullerton, CA). Low-density lipoprotein (LDLc) cholesterol was calculated as seen below. The LDL cholesterol calculation is reliable in the absence of severe hyperlipidemia.

$$LDLc = [Total\_Cholesterol(mmol/L) - HDL\_Cholesterol(mmol/L) - (Triacylglycerol(mmol/L)/2.2)]$$

Body composition measurements

Height (nearest 0.1 cm) and weight (nearest 0.1 kg) measurements were collected and Body Mass Index (BMI) calculated. BMI was defined as weight divided by height squared (kg/m^2). Percent body fat (%BF), percent trunk fat (%TF), and percent android (abdominal) fat (%AF), were measured utilizing dual-energy X-ray absorptiometry (DXA, Lunar Prodigy; GE Medical Systems, Madison, WI). Measurements were performed on subjects in a supine position, after the removal of all metal accessories [91, 208]. Version 12.2 of the enCORE (enCORE,Ver 12.2, 2008, GE Medical Systems, Madison, WI) software package was used for DXA analysis. DXA measurements (fat weight, lean tissue weight and bone weight) are recorded to the nearest 0.001 kg.

Overfeeding protocol

Participants consumed 70% more calories than their normal energy requirements (hypercaloric), which consisted of 15% protein, 35% fat, and 50% carbohydrates to mimic the common daily diet in North America. Baseline energy requirements were determined from three 24-hr diet recalls and a 30-d dietary inventory. The average caloric intake per day was as follows: energy (baseline: 2969 kcal; overfeeding: 5471 kcal), protein (baseline: 106 g; overfeeding: 178 g), carbohydrates (baseline: 394 g; overfeeding: 713 g), fiber (baseline: 19 g; overfeeding: 33 g), total fat (baseline: 105 g; overfeeding: 221 g), saturated fat (baseline: 38 g; overfeeding: 71 g), and cholesterol (baseline: 304 mg; overfeeding: 735 mg). Subjects were offered meals at 0900, 1200, and

1700h every day, and energy values and macronutrient content of the food were measured by using FOOD PROCESSOR SQL software (version 9.5.0.0; ESHA Research, Salem, OR). The actical physical activity monitor (Mini Mitter Co, Inc, Bend, OR) was used to determine the total energy expenditure. Physical activity levels were controlled below 15% between the baseline and overfeeding period. Full details of our overfeeding protocol have been previously described and all of the participants in this study were members of these studies [91, 92,207]. On average, subjects gained 2.2 ± 1.4 kg body weight, of which 44.1 ± 30.4% (0.842 kg ± 0.531) was body fat. A 7-day overfeeding protocol, 70% above normal energy requirements, was chosen for this study to ensure that the intervention would induce metabolic changes[91, 92,207].

Statistical analysis

Data are presented as means ± SDs unless otherwise stated. Before any statistical analysis was performed, data that were not normally distributed were logarithmically transformed (TG, insulin, HOMA-IR and HOMA-β) to approximate normal distribution. Sixty-four young men were used for the analysis of this paper. Subjects were classified on the basis of %BF as normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese ($\geq$ 26%) according to criteria recommended by Bray [3]. Statistical analysis was also performed on adiposity groups classified by BMI according to the WHO criteria [210]. To further explore the relationship between adiponectin and body composition, participants were divided into tertiles according to baseline fasting serum adiponectin concentrations (μg/ml) as follows: Low (Bottom 33.3%), Medium (middle 33.3%) and

High (top 33.3%) adiponectin concentration. The range of serum adiponectin at baseline for the Low, Medium and High adiponectin concentration groups were 1.60-8.15µg/ml, 8.20-13.80µg/ml, and14.20-27.10µg/ml respectively.

Differences in variables between the three adiposity groups in response to overfeeding were analyzed by using a mixed model repeated two-way analysis of variance. Baseline values between the three adiposity groups were analyzed using a one-way analysis of variance. The Bonferroni post-hoc test was run after the one-way ANOVA and the two-way ANOVA displaying a significant overfeeding by adiposity interaction. Within group analysis on the response to overfeeding was performed with a student paired t-test on variables that displayed a significant overfeeding by adiposity interaction effect. Pearson's correlation analyses were performed to screen for potential factors related to fasting adiponectin concentrations followed by partial correlation analyses after controlling for age. Bonferroni testing was applied to correct for multiple comparisons. Two correlation analyses were performed as follows: 1) adiponectin at baseline was compared with all variables at baseline; and 2) adiponectin at baseline was compared with changes in all variables in response to overfeeding to investigate whether baseline adiponectin could predict the changes in related markers. SPSS software version 18.0 (SPSS Inc, Chicago, IL, USA) was used for all analyses. Statistical analyses were 2-sided and $p \leq 0.05$ was considered to be statistically significant.

4.3 - RESULTS

Comparison of characteristics at baseline and in response to short-term overfeeding

Physical and biochemical characteristics of subjects at baseline are shown in **Table 4.1**.Fasting serum adiponectin concentrations at baseline for normal weight (11.60 ± 6.3 µg/ml), overweight (12.84 ± 4.6 µg/ml) and obese (10.69 ± 6.3 µg/ml) subjects were not significantly different from one another. Adiponectin concentrations were also analyzed according to BMI criteria where no significant differences were found (data not shown).

Changes in body composition and phenotypes of glucose metabolism and lipids in response to the 7-day overfeeding are also described in **Table 4.1**.The 7-day positive energy challenge protocol significantly increased subcutaneous body fat, serum lipids (except LDL cholesterol), insulin resistance and β cell function. The only significant overfeeding x adiposity status interactions found from our analysis were for %BF and %TF, indicating that normal weight subjects experienced a greater significant increase in body fat percentage than overweight and obese (**Table 4.1**). The analysis of variance revealed that adiponectin concentrations were significantly increased due to the 7-day positive energy challenge ($p < 0.0001$). Fasting adiponectin levels increased by 18.9% within the entire cohort after overfeeding while increasing by 20.3%, 15.4% and 19.9% among normal-weight, overweight and obese young men respectively. With a ~19% change in adiponectin due to overfeeding with 64 subjects the calculated power was 0.81.

Correlations of adiponectin with phenotypes of glucose and lipid metabolism

Correlations between baseline adiponectin and baseline phenotypes were assessed (**Table 4.2**). In the entire cohort at baseline, adiponectin was negatively correlated with weight and BMI along with a positive correlation with HDLc. However, when these analyses were repeated according to adiposity status no correlation between adiponectin and these variables were found. In addition, when insulin and HOMA-IR were controlled for during partial correlation analysis on the entire cohort the relationship between adiponectin with weight and BMI were nullified. Adiponectin was significantly inversely correlated with %TF, glucose, insulin, and HOMA-IR in the obese group. Correlations between baseline adiponectin and the changes in phenotypes were also assessed to determine if adiponectin could act as a predictor of these parameters under conditions of an energy surplus (**Table 4.3**). Only two significant negative correlations between baseline adiponectin and the change in phenotypes due to overfeeding were found among the adiposity groups. The first inverse relationship was found between adiponectin and insulin in the normal weight group and the second inverse relationship was found between adiponectin and triacyglycerol in the obese group.

Comparison of body composition and lipid profile measures in low, medium and high baseline fasting serum adiponectin concentrations.

Lastly, we wanted to investigate the relationship between low, medium, and high baseline fasting serum adiponectin concentrations and markers for lipids and body composition. The between group comparison at baseline demonstrated that there were no significant differences in body composition measurements among the low, medium and

high adiponectin concentration groups. Total %BF stratified in the low, medium, and high adiponectin concentrations were 23.9 ± 9.4%, 21.9 ± 8.4% and 22.85 ± 6.8% respectively ($p = 0.735$). BMI values at baseline were also not significantly different among the low, medium, and high adiponectin concentrations groups. It would seem that only HDL cholesterol was significantly different between the low and medium ($p = 0.044$) as well as the low and high ($p = 0.01$) baseline adiponectin concentration groups. The two-way ANOVA revealed that the 7-day overfeeding effectively increased whole adiponectin concentrations ($p < 0.0001$) and in addition that there was a significant overfeeding by adiponectin concentration group interaction ($p = 0.002$). Sub-group analysis revealed that adiponectin significantly increased within the low baseline adiponectin group from 5.39 ± 2.0 µg/ml to 9.11 ± 3.75 µg/ml ($p < 0.001$) and the medium baseline adiponectin group from 10.61 ± 1.7 µg/ml to 13.05 ± 3.1 µg/ml ($p < 0.001$), but did not significantly increase within the high baseline adiponectin group (18.24 ± 3.6 µg/ml to 18.71 ± 2.8 µg/ml).

Table 4.1 - Physical and biochemical characteristics of subjects at baseline and in response to 7-days of overfeeding[1]

	Normal Weight[2] (n = 23 - 25)		Overweight[2] (n = 13-14)		Obese[2] (n = 22 - 25)	
	Before	**After**	**Before**	**After**	**Before**	**After**
Age (y)	23.9 ± 3.7	NA	22.0 ± 3.1	NA	23.2 ± 2.4	NA
Height (cm)	178.9 ± 6.6	NA	179.6 ± 4.8	NA	179.6 ± 7.1	NA
Weight (kg) [4,6,7]	72.3 ± 9.6	74.4 ± 9.6	77.8 ± 4.3	79.4 ± 4.3	91.4 ± 15.4	93.9 ± 16.0
BMI (kg/m^2) [4,6,7]	22.6 ± 2.7	23.2 ± 2.9	24.1 ± 1.3	24.6 ± 1.5	28.2 ± 4.2	29.1 ± 4.3
Percent Body Fat (%) [3,6,7,8]	14.82 ± 3.4	15.60 ± 3.4 [9]	22.54 ± 0.8	22.82 ± 1.1 [9]	31.15 ± 4.9	31.01 ± 4.8
Percent Trunk Fat (%) [3,6,7,8]	16.78 ± 3.7	17.78 ± 3.8 [9]	25.39 ± 1.9	25.79 ± 2.2 [9]	35.07 ± 5.4	34.97 ± 5.3
Percent Android Fat (%) [3,6,7]	19.38 ± 4.4	20.16 ± 5.0	28.84 ± 2.6	29.45 ± 2.7	40.00 ± 7.2	40.77 ± 6.8
Total Cholesterol (mmol/L) [6]	4.41 ± 0.9	4.68 ± 0.9	4.63 ± 0.9	4.72 ± 1.1	4.59 ± 0.7	4.86 ± 0.8
HDL-Cholesterol (mmol/L) [6]	1.38 ± 0.3	1.48 ± 0.3	1.38 ± 0.3	1.43 ± 0.3	1.12 ± 0.2	1.34 ± 0.3
LDL-Cholesterol (mmol/L)	2.61 ± 0.7	2.64 ± 0.7	2.82 ± 0.7	2.83 ± 0.9	2.81 ± 0.7	2.83 ± 0.6
Triacylglycerol (mmol/L) [6,7]	0.94 ± 0.3	1.22 ± 0.8	0.92 ± 0.3	1.00 ± 0.5	1.35 ± 0.7	1.57 ± 0.9
Glucose (mmol/L)	4.98 ± 0.4	5.03 ± 0.5	5.03 ± 0.4	5.09 ± 0.6	5.24 ± 0.7	5.11 ± 0.5
Insulin (pmol/L) [4,6,7]	44.22 ± 23.8	64.02 ± 23.7	51.54 ± 17.03	68.69 ± 43.6	80.19 ± 53.3	108.22 ± 76.7
HOMA-IR [4,6,7]	1.43 ± 0.8	2.09 ± 0.9	2.35 ± 2.68	2.95 ± 2.9	2.80 ± 2.3	3.67 ± 2.8
HOMA-β [4,6,7]	85.45 ± 39.38	126.34 ± 49.9	101.49 ± 25.1	140.84 ± 101.7	134.95 ± 63.0	190.28 ± 107.3
Adiponectin (µg/mL) [6]	11.60 ± 6.3	13.96 ± 4.5	12.84 ± 4.6	14.81 ± 4.05	10.69 ± 6.3	12.82 ± 6.1

[1] All values are means ± SDs. HOMA - IR, Homeostasis model assessment of insulin resistance; HOMA - β of β cell function; NA, not applicable. Adiposity status and response to overfeeding analyzed by 2 - factor mix model ANOVA (SPSS, version 17.0 Chicago, IL, USA) for repeated measures.

[2] Subjects were classified on the basis of %BF as either normal weight (8 - 20.9%), overweight (21 - 25.9%) or obese (> 26%) according to criteria recommended by Bray.

[3] Significant difference between normal weight, overweight and obese subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[4] Significant difference between normal weight vs obese subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[5] Significant difference between obese vs normal weight and obese vs overweight subjects at baseline (1 - Way ANOVA, followed by a Bonferroni corrected test, $P < 0.05$).

[6] Significant difference due to overfeeding (2 - Way mixed model ANOVA, $P < 0.05$).

[7] Significant difference due to adiposity status (2 - Way mixed model ANOVA, $P < 0.05$).

[8] Significant overfeeding by adiposity status interaction (2 - Way mixed model ANOVA, followed by a Bonferroni corrected test when significant, $P < 0.05$).

[9] Significant difference within group (paired *t*-test, $P < 0.05$).

Table 4.2 - Partial correlations of baseline variables related to baseline fasting serum adiponectin, after control for age[1]

	All Subjects (n = 59 - 64)		Normal weight[2] (n = 23 - 25)		Overweight[2] (n = 14)		Obese[2] (n = 22 - 25)	
	r	p	r	p	r	p	r	p
Weight	- 0.246	0.050[3]	-0.284	NS	-0.425	NS	-0.177	NS
BMI	- 0.289	0.023[3]	-0.322	NS	-0.188	NS	-0.296	NS
Percent Body Fat	- 0.127	NS	-0.094	NS	0.338	NS	-0.309	NS
Percent Trunk Fat	- 0.155	NS	-0.096	NS	0.235	NS	-0.403	0.041[3]
Percent Android Fat	- 0.140	NS	0.064	NS	0.066	NS	-0.343	NS
Total Cholesterol	0.057	NS	0.340	NS	0.050	NS	-0.313	NS
HDL-Cholesterol	0.401	0.001	0.178	NS	0.222	NS	0.357	NS
LDL-Cholesterol	0.020	NS	0.328	NS	-0.039	NS	-0.242	NS
Triacylglycerols	-0.175	NS	0.089	NS	0.167	NS	-0.339	NS
Glucose	-0.225	NS	0.196	NS	0.026	NS	-0.427	0.042[3]
Insulin	-0.225	NS	0.126	NS	-0.192	NS	-0.499	0.015[3]
HOMA-IR	-0.229	NS	0.141	NS	-0.170	NS	-0.505	0.014[3]
HOMA-Beta	-0.204	NS	0.042	NS	-0.292	NS	-0.399	NS

[1] HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for potential factors related to fasting adiponectin.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

Table 4.3 - Partial correlations of changes in variables due to overfeeding related to baseline fasting serum adiponectin, after control for age[1]

	All Subjects (n = 59 - 64)		Normal weight[2] (n = 23 - 25)		Overweight[2] (n = 14)		Obese[2] (n = 22 - 25)	
	r	p	r	p	r	p	r	p
Weight	-0.043	NS	0.015	NS	-0.103	NS	-0.049	NS
BMI	-0.045	NS	0.011	NS	-0.103	NS	-0.050	NS
Percent Body Fat	0.066	NS	0.155	NS	-0.415	NS	-0.027	NS
Percent Trunk Fat	0.101	NS	0.158	NS	-0.248	NS	0.183	NS
Percent Android Fat	-0.123	NS	-0.191	NS	-0.244	NS	-0.057	NS
Total Cholesterol	0.053	NS	0.146	NS	0.326	NS	-0.069	NS
HDL-Cholesterol	-0.086	NS	0.050	NS	-0.073	NS	-0.115	NS
LDL-Cholesterol	0.168	NS	0.241	NS	0.439	NS	-0.040	NS
Triacylglycerols	-0.142	NS	-0.035	NS	-0.430	NS	-0.435	0.038[3]
Glucose	-0.045	NS	-0.264	NS	0.095	NS	0.002	NS
Insulin	-0.125	NS	-0.438	0.032[3]	0.313	NS	0.112	NS
HOMA-IR	-0.084	NS	-0.175	NS	0.059	NS	0.230	NS
HOMA-Beta	-0.081	NS	-0.298	NS	0.210	NS	0.099	NS

[1] HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function. Partial correlation analysis after control for age was used to screen for the potential changes in factors due to overfeeding related to fasting adiponectin.

[2] Subjects were classified on the basis of %BF as either normal weight (8 – 20.9%), overweight (21 – 25.9%) or obese (> 26%) according to criteria recommended by Bray.

[3] Not significant after Bonferroni correction to adjust for the multiple variables tested.

4.4 - DISCUSSION

The noteworthy finding of the present investigation is that circulating adiponectin levels are significantly increased by a short-term positive energy challenge independent of adiposity status. Taking into consideration that the majority of human cross-sectional studies show that circulating adiponectin is diminished among the obese population [119, 122-124], and significantly promoted after weight reduction [124, 125, 128, 129], the increase in circulating adiponectin we observed after weight gain in overweight and obese subjects was unexpected. White adipose tissue being the primary location for the adiponectin production, together with the paradoxical inverse relationship between adiponectin and obesity, advocates that circulating adiponectin may be regulated by an adiposity feedback inhibition [124]. Rodent studies, observing a suppression of adiponectin in obese and ob/ob mice [127, 130,131], support the notion of an adiposity regulated feedback mechanism. However adiponectin transgenic ob/ob mice effectively ameliorating insulin resistance but not obesity [132, 133] and adiponectin knockout mice developing insulin resistance without affecting weight or adiposity [126, 134], provides the strongest evidence that adiponectin is not mediated by adiposity alone. In addition, the administration of adiponectin to ob/ob, db/db, and adiponectin knockout mice can ameliorate the development of insulin resistance without significantly influencing adiposity [127, 135]. In the present study adiponectin concentration significantly increased within the entire cohort and the normal-weight, overweight, and obese subgroups due to overfeeding. However, there was no significant difference in the increase of circulating adiponectin between the three obesity groups due to overfeeding.

To further explore this unique finding we stratified our subjects into a low, medium and high adiponectin concentration groups to determine whether adiponectin concentration was associated with the increase in adiposity due to the positive energy challenge. Consequently, there was no significant difference in the increase of our adiposity measurements among adiponectin concentration groups before or after overfeeding. These findings would suggest that there is a definite capacity, regardless of obesity status, for the human body to significantly synthesize and secrete more adiponectin into circulation after being exposed to a short-term overfeeding. To our knowledge this is the first study of its kind to demonstrate that adiponectin levels will significantly increase after a short-term positive energy challenge independent of obesity status.

Our laboratory has demonstrated that a short-term positive energy challenge can significantly affect both physical and biochemical obesity-related phenotypes [91, 92,207]. However, to date, very few other overfeeding studies have been performed due to the inherent difficulty to carry out this type of study. One 5-day high-fat (60% fat, 32.5% carbohydrates and 7.5% protein) overfeeding (50% greater than normal caloric intake) investigation on 26 normal-weight young men observed a significant increase in adiponectin concentration [84]. Although the age and body weight of these subjects were comparable to the normal-weight group from our current study, the overfeeding intervention and diet composition were considerably different. This study also did not contain any overweight and/or obese subjects. Nevertheless our findings, with those of Bron et al. [84], provide evidence that the significant increase of adiponectin due to

overfeeding is likely driven by an increase in total caloric intake rather than macronutrient composition. A 4-week positive energy challenge on 18 young normal-weight adults (12 male and 6 female) did not induce a significant increase in circulating adiponectin [85]. Although no explanation was provided, low statistical power due to the small number of subjects could be a potential reason. Finally, a long-term (100-day) overfeeding study investigated the influence of chronic food intake on circulating adiponectin in 12 pairs of young male identical twins [93]. Study participants were overfed by 840 kcal for 100 days and adiponectin concentration was found to have decreased. However there was no relationship between baseline adiponectin and adiposity before or after prolonged overfeeding. A replication study would be favored since this is the only long-term positive energy challenge study containing a fairly small sample size. Overall, the current human overfeeding studies demonstrate that adiponectin concentrations are up regulated during a short-term positive energy challenge. Our findings would suggest that adiponectin plays a protective role during obesity development and its role is more potent during the early stages of the chronic positive energy balance. This assumption is supported by data from an animal experiment which found that circulating adiponectin concentrations significantly increased in C57BL/6J mice on a high-fat diet during the initial stages of adiposity development [221] and decreased after the full 18 week diet.

The present investigation did not find a significant difference in baseline adiponectin concentration among normal-weight, overweight and obese subjects nor were

any associations found between adiponectin concentration and adiposity measurements for these groups. In addition, no significant difference was observed for any body composition measurement among the low, medium and high adiponectin concentration groups. The data from this investigation does not support the hypothesis that adiposity status is a strong contributing factor to circulating adiponectin concentration. We did observe that circulating adiponectin was inversely correlated with BMI and weight for the entire cohort along with %TF in obese subjects. However, recent studies have advocated that the inverse relationship between adiponectin and adiposity phenotypes is solely dependent upon the development of insulin resistance [116, 120, 123,139]. Therefore, after controlling for fasting insulin and/or HOMA-IR in our analysis the significant partial correlation between adiponectin and aforementioned body composition measurements were nullified. Therefore, our data supports the hypothesis that the paradoxical relationship between adiposity and circulating adiponectin is not dependent upon the accumulation of adipose tissue but rather the concomitant increase in insulin resistance during obesity development [120, 121, 139].

The primary limitation of the present study is that we only studied young males (19-29 yr), which limits the application of our conclusions to other groups. Larger population studies investigating the effects of overfeeding on a wider age distribution which includes females is warranted. In addition, only total circulating adiponectin was measured in this investigation. It has been suggested that high molecular weight adiponectin is the primary physiologically active isoform [222] of adiponectin. However,

it has also been shown that low, medium and high molecular weight adiponectin respond to nutritional regulation and exercise the same as total circulating adiponectin [128]. In addition, the influence of inflammatory markers (such as TNF-α, IL-6 and hs-CRP, etc.) on adiponectin regulation is a noteworthy consideration [223]. Therefore, future short-term overfeeding studies investigating influence of inflammatory markers on the regulation and circulation of adiponectin in adipose tissue and peripheral circulation are warranted.

In summary, we investigated the response of circulating adiponectin to a 7-day positive energy challenge mimicking a North American diet in 64 healthy young men. The relationship between adiponectin and adiposity status was also analyzed. The current study revealed that endogenous adiponectin concentration significantly increased within the entire cohort and all three adiposity groups in response to short-term overfeeding. Fasting serum adiponectin levels were found to be similar among normal-weight, overweight and obese young men at baseline and were not associated with adiposity. Negative correlations of adiponectin with %TF, body weight and BMI was observed, but likely arbitrated to insulin resistance. Our data suggests for first time that the increase in adiponectin, in the face of short-term positive energy challenge, may act as a protective mechanism during periods of weight gain against insulin resistance independent of adiposity status and diet composition.

5

Chapter 5: High Dietary Magnesium Intake is Associated with Low Insulin Resistance in the Newfoundland Population.

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5.1 - INTRODUCTION

Type 2 diabetes comprises 90% of all diabetic cases and has become an ever increasing healthcare challenge as the number of people affected reaches epidemic proportions [1]. The prevalence of this condition is expected to reach over 438 million people globally by the year 2030 and carries with it a significant fiscal burden [145]. This is especially relevant to the Canadian province of Newfoundland and Labrador considering it has one the highest rates of diabetes and obesity in Canada. Currently, 9% of the population of Newfoundland and Labrador struggle with diabetes which corresponds to an annual cost of $254 million dollars [11]. The Canadian Diabetes Association has projected that by the year 2020 over 15% of Newfoundland and Labradorean's will be diabetic with an annual health care cost exceeding $360 million dollars [11]. Although there is currently no medical intervention capable of preventing the development of diabetes, simple lifestyle modifications (such as increased physical activity, moderate weight loss, and eating behavior modifications) have been shown to attenuate the onset of type 2 diabetes [1, 146]. However few studies to date have investigated the association between dietary micronutrient intake and insulin function in the general population.

Magnesium, a cofactor required in over 300 enzymatic reactions, is the fourth most abundant cation in the human body involved with both glucose metabolism and insulin homeostasis [140, 141]. Although recent evidence has suggested that dietary magnesium intake may play an important role in enhancing insulin sensitivity, population

based studies have found conflicting evidence regarding the potential benefit of dietary magnesium intake. Several studies have correlated low dietary magnesium intake [147-149] and serum magnesium [224] with increased insulin resistance [150-152]. However, other studies do not support the proposed protective effect that dietary magnesium can attenuate the development of diabetes [154-156]. Of note, most studies have not adequately controlled for adiposity in their analysis. Instead of controlling for relative body fat, the majority of studies have utilized the body mass index (BMI) which cannot accurately distinguish fat mass from and fat-free mass [16]. Since adiposity is the parameter most closely linked with the development of insulin resistance [157], controlling for this important confounding factor is critical. Therefore we designed the present study to investigate the association between magnesium intake and insulin resistance in the Canadian province of Newfoundland and Labrador, taking into consideration age, gender, caloric intake, physical activity, medication use, smoking status, menopause and adiposity.

5.2 - SUBJECTS & METHODS

Ethics Statement

Ethics approval was obtained from the Human Investigation Committee, Faculty of Medicine, Memorial University, St. John's, Newfoundland, Canada. All subjects provided written and informed consent before participation in this study.

Subjects

All 2330 subjects (600 men 1725 women) from the current study are volunteers from the general population of Newfoundland and Labrador and the cohort for our ongoing CODING (Complex Diseases in the Newfoundland Population: Environment and Genetics) Study. The CODING study is a large-scale nutrigenomics investigation [15, 16,225]. Eligibility of participants for the CODING study are based upon the following inclusion criteria: 1) $\geq$ 19 yrs of age; 2) at least a third generation Newfoundlander; and 3) healthy, without any serious metabolic, cardiovascular, or endocrine diseases. The primary method of subject recruitment for the CODING study was the use of posters and handouts. This literature was distributed throughout public facilities (offices, hospitals, and gyms) in the city of St. John's, Newfoundland and Labrador. All subjects completed screening questionnaires providing information about physical characteristics, physical activity, health status, and dietary practices. Anthropometrics, body composition, and biochemical measurements were collected following a 12 hour fast.

Physical Activity

Physical activity patterns were measured using the ARIC-Baecke Questionnaire, which consists of a Work Index, Sports Index, and Leisure Time Activity Index [226].

Dietary magnesium assessment

Dietary intake patterns of each participant were assessed using a 124 item semi-quantitative Willett Food Frequency Questionnaire (FFQ) [227]. The Willett FFQ is one of the most commonly used dietary questionnaires for large-scale epidemiologic studies [228]. The Willett FFQ obtains from subjects, the number of weekly servings consumed of specific food item(s). NutriBase Clinical Nutrition Manager (version 8.2.0; Cybersoft Inc, Phoenix, AZ) software package was used to convert weekly serving values into mean daily serving values to calculate the total daily intake of magnesium(mg/day) for each individual. The nutritional information, including dietary magnesium intake, was computed for all subjects in the CODING study [225].

Anthropometric and body composition measurements

Subjects were weighed (Health O Meter, Bridgeview, IL) to the nearest 0.1 kg in standardized clothing (hospital gown). Height was measured using a fixed stadiometer (nearest 0.1 cm). Body mass index (kg/m^2) was calculated as weight in kilograms divided by participants' height in meters squared. Whole body composition measurements including fat mass, lean body mass, and bone mineral densities were measured using dual-energy X-ray absorptiometry (DXA) Lunar Prodigy (GE Medical Systems, Madison, WI). DXA can produce an accurate measurement of adipose tissue within the body with a low margin of error. For this reason, DXA is considered to be one of the most accurate measurements of adiposity. DXA measurements were performed on subjects following the removal of all metal accessories, while lying in a supine position

as previously described by us [15, 16, 225]. Body fat percentage (%BF) is determined as a ratio of fat mass to total body mass (Version 12.2 of the enCORE software). Quality assurance was performed on our DXA scanner daily and the typical CV was 1.3% during the study period.

Biochemical measurements

Serum concentrations of glucose and magnesium were measured on an Lx20 analyzer (Beckman Coulter Inc., Fullerton, CA) using Synchron reagents. Serum insulin was measured on an Immulite Immunoassay analyzer. Insulin resistance and beta cell function were determined with the homeostasis model assessment (HOMA), as described by Matthews et al [213].

$$\text{HOMA-IR} = [(\text{Fasting Insulin (mU/L)} \times \text{Fasting Glucose (mmol/L)}))/22.5]$$

$$\text{HOMA-}\beta = [(20\times \text{Fasting Insulin (mU/L)}) / (\text{Fasting glucose (mmol/L)} - 3.5)]$$

Smoking, Medication and Menopausal Status

A self-administered screening questionnaire was used to collect information about the subjects' demographics, personal and family medical history, medication use (yes or no), and smoking status (yes or no). Women completed an additional questionnaire regarding menstrual history and menopausal status (pre- or post-menopausal) [16, 225].

Statistical analysis

All data are reported as mean ± standard deviation (SD) unless otherwise stated. Participants with daily total caloric intake (kcal/day) falling outside the range of ± 3SDs were considered outliers and excluded from further analyses to account for possible errors associated with over- or under-reporting of food intake on the FFQ. Insulin, HOMA-IR, and HOMA-β were log-transformed to normalize distributions and meet the assumptions of statistical tests. The sample size for the study was 2295 participants (590 men, 1705 women). Differences in physical, biochemical, and dietary patterns between men and women were assessed using one-way ANOVA. Subjects were subdivided by adiposity into Normal-Weight (NW), Overweight (OW), and Obese (OB) groups based on %BF measured by DXA according to the age and gender-specific criteria recommended by Bray [3]. Differences in physical, biochemical, and dietary patterns among adiposity groups was assessed using a one-way ANCOVA controlling for age, gender, total caloric intake, physical activity, medication use and smoking and menopause. As the number of underweight subjects was too low (n=28) to perform effective statistical analysis, they were excluded from these analyses. To initially explore the relationship between dietary magnesium and IR (fasting glucose (mmol/l), insulin (pmol/l), HOMA-IR, HOMA-β), participants were divided into a tertile (low, medium, or high) based upon dietary magnesium intake (mg/day) which were assessed using an ANCOVA controlling for age, gender, total caloric intake, physical activity, medication use, smoking, menopause and %BF. Subsequently, multiple regression analyses were used to more rigorously explore the potential association of dietary magnesium intake (g/kg body weight) with insulin resistance (HOMA-IR) within the entire cohort and

among men and women adjusting for age, gender, caloric intake, physical activity, medication use, smoking, menopause and %BF or BMI. However, only caloric intake, physical activity, medication use, menopause and %BF or BMI were statistically considered as confounding variables within the regression models. In addition, due to the significant interaction of adiposity and menopause on the association between dietary magnesium intake and insulin resistance, multiple regression analysis was performed among normal-weight, overweight and obese subjects along with pre-menopausal (n = 834) and post-menopausal (n = 577) women. Multiple regression analyses were performed on subjects stratified into tertiles (low, medium, or high) based upon both BMI and %BF measured from DXA to assess the difference in their adjustments on the relationship between dietary magnesium intake and insulin resistance. Lastly, Binary logistic analysis was also performed to explore the association between magnesium intake (g/kg/day) and diabetes status. Diabetes status was defined by a fasting glucose cutoff (glucose > 7.0 mmol/L) together with those individuals who reported they were diabetics. Under the aforementioned definition, 102 subjects were labeled as diabetics for this analysis. Age, gender, caloric intake, physical activity, medication use, menopausal status, and smoking status were used as covariates. However, only caloric intake, medication status and age were statistically considered as confounding variables within the binary logistic model. Some subjects had missing data in one or more measurements. Five subjects did not disclose their smoking status and eight subjects did not respond to the medication use question. 1981 participants had all complete dataset for all variables measured to be used for the ANCOVA and multiple regression analysis.

All statistical analyses were performed with and without diabetic subjects; however none of the results were not affected. All statistical analyses were performed using PASW 19.0 (SPSS Inc., Chicago, IL). All tests were two-sided and a *p*-value <0.05 was considered to be statistically significant.

5.3 - RESULTS

Physical, biochemical, and dietary characteristics of normal-weight, overweight, and obese participants

Physical, biochemical, and dietary characteristics for men and women along with those for normal-weight, overweight and obese participants are presented in **Table 5.1**. Glucose, insulin, and HOMA-IR were significantly greater for men than women, but HOMA-β was significantly greater among women. In terms of dietary intake, male participants had a significantly higher total daily caloric and magnesium intake than females. However, women had significantly greater magnesium intake (mg/day/kg) per kilogram body weight than men. Insulin, glucose, HOMA-β, and HOMA-IR values were greater among overweight and obese subjects, which we have previously described [15, 16,225]. Individuals with the highest insulin, HOMA-IR and HOMA-β had the highest levels of adiposity. In addition, subjects with highest levels of adiposity had the lowest levels of magnesium intake (mg/day), and magnesium intake per kilogram of body weight (mg/day/kg). The findings remained significant after controlling for total age, gender, caloric intake, physical activity, medication use, menopausal status and smoking status. Serum magnesium was not significantly different among adiposity groups (**Table**

5.1).

Insulin resistance among low, medium, and high dietary magnesium intake groups

Physical, biochemical, and dietary characteristics were assessed among a tertile (low, medium, or high) based upon dietary magnesium intake (mg/day) (**Table 5.2**). Those individuals with the highest magnesium intake had the lowest insulin, HOMA-IR and HOMA-ß. Individuals with the lowest magnesium intake had the highest fasting insulin levels, HOMA-IR, and HOMA-ß. The findings remained significant after accounting for age, gender, caloric intake, physical activity, medication use, smoking, menopause and %BF. The dietary magnesium intake ranged from; 33.06 to 270.83 mg/day in the low dietary magnesium intake group, 270.84 to 393.66 mg/day in the medium dietary magnesium intake group, and 394.07 to 2493.01 mg/day in the high dietary magnesium intake group. Dietary magnesium intake per kilogram of body weight ranged from; 0.43 to 5.3mg/kg/day in the low dietary magnesium intake group, 2.0 to 8.18 mg/kg/day in the medium dietary magnesium intake group, and 3.17 to 44.84 mg/kg/day in the high dietary magnesium intake group.

Relationship between dietary magnesium and insulin resistance

Unadjusted and adjusted linear regression analysis results of dietary magnesium intake on insulin resistance are shown in **Table 5.3**. There was a significant negative association between dietary magnesium intake (g/day/kg) with HOMA-IR in the entire cohort before and after adjusting for caloric intake, physical activity, medication use,

menopausal status and %BF or BMI. In addition, the negative association between magnesium intake and insulin resistance was greater having adjusted for adiposity (%BF) over adjusting for BMI (**Table 5.3**). Multiple regression analysis of the entire study cohort also revealed a significant interaction of adiposity (%BF & BMI) with the negative association of magnesium intake with insulin resistance. Therefore, multiple regression analysis was performed on NW, OW and OB groups based upon %BF according to the Bray Criteria (**Table 5.3**). Magnesium intake was found to be significantly negatively associated with HOMA-IR among all adiposity groups. This inverse relationship was greater among overweight and obese subjects and pre-menopausal women.

Relationship between dietary magnesium and insulin resistance among low, medium and high %BF and BMI.

Unadjusted and adjusted linear regression analysis results of dietary magnesium intake (g/day/kg) on insulin resistance among low, medium and high BMI and %BF groups are shown in **Table 5.4**. Magnesium intake was increasingly more negatively associated with HOMA-IR the greater the adiposity (%BF) or body mass index (BMI). Moreover, the negative association between dietary magnesium intake and insulin resistance was more prevalent according to a %BF classification than a BMI classification (**Table 5.4**).

Relationship between dietary magnesium and diabetes status

Binary logistic analysis, performed to explore the association between magnesium intake (g/kg/day) with diabetes status, revealed that magnesium intake (g/kg/day) was significantly negatively associated with diabetes status ($n = 102$, Unstandardized $\beta = -499.80$, standard error 79.45, $p < 0.0001$). Caloric intake, medication status and age were statistically considered as confounding variables within the binary logistic model.

Serum magnesium and markers of insulin resistance

The relationship between serum magnesium and insulin resistance was also explored. Although serum magnesium concentration concomitantly increased with dietary magnesium intake, it was not found to be significantly associated with insulin resistance in the Newfoundland population (data not shown).

Table 5.1. Physical, Biochemical, and Dietary Intake Characteristics of Normal-weight, Overweight, and Obese Participants.

		Gender		Percent Body Fat - Bray Criteria			
	Entire Cohort	**Male**	**Female**	**Normal Weight**	**Overweight**	**Obese**	**p**
	(n = 2295)	**(n = 590)**	**(n=1705)**	**(n = 547)**	**(n = 600)**	**(n=781)**	
Age (yr) [3]	43.16 ± 13	41.04 ± 14.4	43.92 ± 12.4	40.96 ± 14.34	45.02 ± 12.53	44.14 ± 12.3	<0.001
Weight (kg) [2]	74.72 ± 16.5	86.86 ± 15.8	70.5 ± 14.5	64.63 ± 10.47	71.22 ± 11.37	86.12 ± 16.53	<0.001
Height (cm) [2]	165.8 ± 8.6	176.09 ± 6.5	162.24 ± 5.9	166.68 ± 8.5	165.32 ± 8.2	165.98 ± 9.0	NS
Waist (cm) [2]	93.07 ± 15	98.68 ± 13.9	91.13 ± 14.9	82.35 ± 9	90.8 ± 10.3	103.68 ± 14.5	<0.001
Hip (cm) [3]	101.66 ± 11.9	100.8 ± 10.4	101.94 ± 12.4	92.76 ± 6.6	99.48 ± 7.2	110.25 ± 11.8	<0.001
Waist-Hip Ratio [2]	0.91 ± 0.1	0.98 ± 0.1	0.89 ± 0.1	0.89 ± 0.1	0.91 ± 0.1	0.93 ± 0.1	<0.001
BMI (kg/m^2) [2]	27.1 ± 5.2	27.98 ± 4.6	26.79 ± 5.4	23.17 ± 2.6	25.97 ± 2.9	31.19 ± 5.0	<0.001
Total Body Fat (%) [3]	35.17 ± 9.1	26.31 ± 7.7	38.22 ± 7.5	26.86 ± 6.5	34.86 ± 5.7	42.12 ± 6.6	<0.001
Glucose (mmol/L) [2]	5.16 ± 0.9	5.35 ± 1	5.09 ± 0.9	4.98 ± 0.7	5.15 ± 0.8	5.33 ± 1.1	<0.001
Insulin (pmol/L) [2]	68.86 ± 67.7	73.53 ± 63	67.2 ± 69.3	50.58 ± 59.3	64.16 ± 72.8	84.74 ± 73.2	<0.001
HOMA-IR [2]	2.39 ± 3.3	2.65 ± 3.4	2.3 ± 3.3	1.72 ± 3.4	2.21 ± 3.6	3.0 ± 3.4	<0.001
HOMA-β [3]	133.99 ± 165	127.24 ± 129.4	136.25 ± 175.8	111.98 ± 176.3	122.75 ± 102.65	153.38 ± 204.4	<0.001
Magnesium intake (mg/day) [2]	368.57 ± 210.4	394.29 ± 228.7	359.66 ± 203.3	415.96 ± 253.78	354.06 ± 171.3	351.5 ± 208.7	0.003
Magnesium intake (mg/day/kg) [3]	5.15 ± 3.1	4.68 ± 2.8	5.3 ± 3.26	6.51 ± 3.9	5.05 ± 2.5	4.2 ± 2.4	<0.001
Serum Magnesium (mmol/L) [2]	0.88 ± 0.1	0.9 ± 0.09	0.87 ± 0.08	0.88 ± 0.08	0.88 ± 0.08	0.87 ± 0.08	NS
Calories (kcal/day) [2]	1985.8 ± 878.6	2246.5 ± 982.7	1896.1 ± 821.7	2118.6 ± 937.1	1885.9 ± 764.62	1958 ± 897.4	<0.001

[1.] Data presented as mean ± SD. Homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β). Gender differences were assessed with a one-way ANOVA. Subjects were also stratified into normal-weight, overweight and obese based upon %BF according to the Bray criteria. Adiposity differences were assessed with an ANCOVA controlling for caloric intake, physical activity, medication use, and menopause.

[2.] Significantly greater for men compared to women.

[3.] Significantly greater for women compared to men.

[4.] Statistical significance for one-way ANOVA and ANCOVA were set to $p < 0.05$ (IBM SPSS Statistics 19).

Table 5.2. Physical, Biochemical, and Dietary Intake Characteristics According to Magnesium Intake.

	Dietary Magnesium Intake (mg/day)			
	Low Mg Intake	**Medium Mg Intake**	**High Mg Intake**	**p**
	(n=765)	**(n=765)**	**(n=765)**	**-**
Age (yr)	43.78 ± 12.1	43.49 ± 12.3	42.22 ± 14.5	-
Weight (kg)	74.63 ± 16.7	74.46 ± 15.9	75.04 ± 16.8	NS
Height (cm)	164.7 ± 8.2	165.65 ± 8.3	167.07 ± 9.0	NS
Waist (cm)	94.3 ± 15.0	92.94 ± 15.4	91.93 ± 14.5	NS
Hip (cm)	102.99 ± 11.8	101.78 ± 11.9	100.18 ± 11.9	NS
Waist-Hip Ratio	0.91 ± 0.08	0.91 ± 0.08	0.92 ± 0.09	NS
BMI (kg/m^2)	27.4 ± 5.3	27.08 ± 5.1	26.79 ± 5.2	NS
Total Body Fat (%)	36.68 ± 8.3	35.3 ± 9.1	33.5 ± 9.7	-
Glucose (mmol/L)	5.18 ± 0.8	5.18 ± 1.0	5.17 ± 1.0	NS
Insulin (pmol/L)	72.82 ± 68.6	71.45 ± 91.3	60.57 ± 42.9	<0.001
HOMA-IR	2.49 ± 2.5	2.59 ± 5.2	2.07 ± 1.8	0.003
HOMA-β	142.37 ± 213.3	135.7 ± 182.0	116.24 ± 89.9	<0.001
Magnesium intake (mg/day)	201.57 ± 48.6	328.85 ± 36.3	575.98 ± 224.1	<0.001
Magnesium intake (mg/day/kg)	2.83 ± 0.9	4.59 ± 1.0	7.99 ± 3.6	<0.001
Serum Magnesium (mmol/L)	0.88 ± 0.08	0.88 ± 0.07	0.89 ± 0.08	0.023
Calories (kcal/day)	1299.11 ± 409.9	1904.21 ± 440.42	2747.22 ± 928.49	-

[1.] Data presented as mean ± SD. Homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β).

[2.] Subjects were stratified into a tertile (low, medium and high) based upon magnesium intake (mg/day).

[3.] Magnesium intake group differences were assessed with an ANCOVA controlling for caloric intake, physical activity, medication use, menopause and %BF.

[4.] Statistical significance for one-way ANCOVA was set to $p < 0.05$ (IBM SPSS Statistics 19).

Table 5.3. Regression Models of Magnesium Intake on Insulin Resistance.

	Unadjusted			Adjusted			Adjusted + %BF			Adjusted + BMI		
	β	**β***	**p**	**β**	**β***	**p**	**β**	**β***	**p**	**β**	**β***	**p**
HOMA-IR												
Entire Cohort	-21.03 (1.8)	-0.232 (0.02)	< 0.0001	-33.75 (2.9)	-0.374 (0.03)	< 0.0001	-19.33 (2.9)	-0.214 (0.03)	< 0.0001	-11.65 (2.9)	-0.129 (0.03)	< 0.0001
Normal-weight	-7.68 (2.6)	-0.085 (0.03)	0.003	-10.92 (4.0)	-0.120 (0.05)	< 0.0001	-	-	-	-	-	-
Overweight	-12.84 (3.9)	-0.141 (0.04)	0.001	-30.13 (6.9)	-0.332 (0.08)	< 0.0001	-	-	-	-	-	-
Obese	-23.79 (4.0)	-0.262 (0.04)	< 0.0001	-45.97 (6.5)	-0.506 (0.07)	< 0.0001	-	-	-	-	-	-
Pre-Menopause	-15.07 (2.5)	-0.188 (0.03)	< 0.0001	-35.32 (4.4)	-0.439 (0.05)	< 0.0001	-20.4 (4.6)	-0.254 (0.05)	< 0.001	-13.57 (4.4)	-0.169 (0.05)	0.002
Post-Menopause	-18.91 (3.5)	-0.208 (0.04)	< 0.0001	-27.44 (4.6)	-0.311 (0.05)	< 0.0001	-16.89 (4.8)	-0.191 (0.05)	<0.001	-7.32 (4.6)	-0.083 (0.05)	NS

[1.] Regression model adjusted for caloric intake, physical activity, medication use and menopausal status.

[2.] β = Unstandardized Beta (standard error) , β* = Standardized Beta (standard error), Magnesium intake (g/day/kg).

[3.] Normal-weight, overweight and obese groups are based upon %BF according to the Bray criteria.

[4.] Magnesium intake (Pre-Menopause 360.63 ± 209.8 mg/day, Post-Menopause 353.82 ± 192.9 mg/day)
(Entire cohort, Normal-weight, Overweight, & Obese – See Table .1)

[5.] Statistical significance was set to $p < 0.05$ (IBM SPSS Statistics 19).

Table 5.4. Regression Models of Magnesium Intake on Insulin Resistance based upon %BF and BMI.

	Body Fat Percentage								
	Low			**Medium**			**High**		
	β	**β***	**p**	**β**	**β***	**p**	**β**	**β***	**p**
Entire Cohort									
Unadjusted	-14.98 (2.5)	-0.165 (0.03)	< 0.0001	-17.36 (3.4)	-0.191 (0.04)	< 0.0001	-20.98 (4.1)	-0.231 (0.05)	< 0.0001
Adjusted	-14.30 (4.1)	-0.157 (0.04)	0.001	-23.39 (6.0)	-0.258 (0.07)	0.0002	-45.59 (6.48)	-0.502 (0.07)	0.000
	Body Mass Index								
Entire Cohort									
Unadjusted	-3.89 (2.1)	-0.043 (0.02)	NS	-12.4 (3.5)	-0.137 (0.04)	0.0004	-24.27 (4.7)	-0.267 (0.05)	< 0.0001
Adjusted	-9.97 (3.5)	-0.110 (0.04)	0.004	-12.8 (6.4)	-0.141 (0.07)	0.047	-57.6 (7.9)	-0.446 (0.08)	0.000

[1.] Regression model adjusted for caloric intake, physical activity, medication use and menopausal status. Subjects were also stratified into a tertiles(Low, Medium and High) based upon %BF and BMI.

[2.] β = Unstandardized Beta (standard error) , β* = Standardized Beta (standard error), Magnesium intake (g/day/kg).

[3.] Magnesium intake (Low BMI 409.78 ± 243.5 mg/day, Medium BMI 353.24 ± 180.9 mg/day, High BMI 342.76±196.1 mg/day)
(Low %BF 387.5 ± 230.3 mg/day, Medium %BF 360.54 ± 187.5 mg/day, High %BF 357.68 ± 210.7 mg/day)

[4.] Statistical significance was set to $p < 0.05$ (IBM SPSS Statistics 19).

5.4 - DISCUSSION

The noteworthy finding of the present investigation was a beneficial dose dependent relationship between dietary magnesium intake and insulin resistance, independent of age gender, total caloric intake, physical activity, medication use, menopause, and adiposity. However, this favorable association was more significant in overweight and obese subjects suggesting that this population may be more sensitive to the beneficial effects of dietary magnesium intake. Obesity, as a disorder, is a well-known condition which can place individuals at a significantly elevated risk for impaired insulin action [144] and various metabolic abnormalities such as hypertension, dyslipidemia, and a reduction in glucose tolerance [144]. The functional relationship between various hormones and obesity-related conditions is a significant focus of obesity research, however diet composition has become increasingly recognized and studied [145, 146]. One study specifically demonstrated that an increase in dietary magnesium can significantly improve insulin sensitivity [153]. Magnesium has been proposed to be functionally related to glucose metabolism through an interaction with tyrosine-kinase activity on the insulin receptor which is associated with the development of insulin resistance and type 2 diabetes [142]. Studies have suggested that the effect of dietary magnesium on decreasing markers of IR [150, 151] and development of type 2 diabetes is more pronounced in overweight patients [150, 151]. However, body fat percentage was not measured in these studies. This is an especially important point to consider when the relative amount and distribution of adipose tissue, both important determinants of insulin sensitivity, cannot be determined by the body mass index [158-160].

In fact the majority of studies evaluating the association between dietary magnesium and insulin resistance have only utilized BMI when attempting to control for adiposity, bearing in mind that percent body fat and BMI likely represent different physiological entities. Having adjusted for the influence of %BF and BMI on the association of dietary magnesium intake with insulin resistance, we observed that this inverse association was stronger after having adjusted for %BF than for BMI which further supports our hypothesis. Considering that our laboratory and others have revealed that BMI is not an accurate measure of body fat due to its inability to differentiate fat mass from fat free mass [15, 186, 229], it is implicit that BMI cannot represent adiposity. We found that overweight and obese individuals, defined by a high resolution adiposity measurement, are more sensitive to the beneficial effect of magnesium intake on insulin resistance. Our current findings, taken together with others of others [150, 151] provides strong evidence that overweight individuals, classified by either %BF or BMI, could potentially benefit from an increase in magnesium intake. It is possible that overweight and obese individuals are better able to absorb and metabolize magnesium, thereby enhancing its action at the insulin receptor and promoting insulin sensitivity. To our knowledge, this study is the first large cross-sectional study to systematically control for major confounding factors including %BF when analyzing the relationship between dietary magnesium intake and markers of insulin resistance. This study is also the first investigation to observe that dietary magnesium is more strongly associated with insulin resistance when adjusting for %BF than BMI.

The association between dietary magnesium intake and insulin resistance was examined with regards smoking [230], menopause [151]and medication [231, 232] as possible confounding factors for this relationship. Evidence suggests that nicotine intake may increase insulin resistance, however we did not find a significant association of smoking status with HOMA-IR or magnesium intake. In addition, during the regression model development smoking status failed to reach statistical significance and was not included in the regression model. Our data would suggest that smoking is not a critical confounding factor regarding the beneficial effect of dietary magnesium intake on insulin resistance in the Newfoundland population. However, this finding may be due to the significantly smaller sample size of smokers (n = 222) to non-smokers (n = 1754) in our study cohort. Further study is needed to elucidate the potential interaction of smoking on the relationship of dietary magnesium intake and insulin resistance. Medication status was significantly associated with insulin resistance and there is evidence to suggest that various medications inhibit magnesium re-absorption in the kidney which can result in magnesium deficiency [231, 232]. However, we were unable to find a significant interaction of medication use on the inverse association with magnesium intake and insulin resistance. Pre-menopausal women were more significantly associated the beneficial effects of magnesium intake on insulin resistance than post-menopausal women. This finding is strengthened by the Shanghai Women's Health Study, designed to assess the prospective risk of type 2 diabetes, which found a statistically significant negative correlation between magnesium intake and type 2 diabetes risk in pre-menopausal women only [151]. We considered, since over 68% of our post-menopausal

women are medication users, that the lack of association of magnesium intake with insulin resistance could be drug related interference. However, this relationship remained absent among post-menopausal women whether or not medication status was included in the regression model. Therefore, our data would suggest that the beneficial effects of magnesium intake are less sensitive among post-menopausal women and further study is needed to explore the physiological mechanism involved. Lastly, we discovered that magnesium intake was significantly negatively associated with diabetes. Considering that insulin resistance is a significant clinical symptom of diabetes and that insulin resistance was significantly inversely associated with magnesium intake this, finding was not surprising. Our results suggest that the potential beneficial influence of magnesium intake exists in a wide range of populations from the general population to insulin resistance and to those people struggling with diabetes in Newfoundland and Labrador.

Aside from investigating the influence of %BF and BMI on the association between magnesium intake and insulin resistance in the general population and the influence that an adiposity status defined by %BF has on this relationship, we also investigated whether an adiposity status defined by BMI would have a different effect than %BF on this association. Considering that the adiposity status criteria for the World Health Organization (WHO) is considerably different from that developed by Bray et al. [3], the association between dietary magnesium intake and insulin resistance was examined among low, medium and high tertiles according to %BF and BMI. Our data revealed that the inverse relationship between dietary magnesium intake and insulin

resistance was progressively stronger the greater the %BF or BMI. However, the aforementioned association was more significantly pronounced for concomitant increases in %BF over BMI. Consider that %BF is a more direct measure of adiposity than BMI, together with our current findings, we recommend that %BF be used when considering adiposity as a factor.

The apparent protective role of magnesium on IR and type 2 diabetes has not been fully explained but is likely due to enhanced insulin sensitivity through multiple mechanisms. For example, phosphorylation of the tyrosine kinase enzyme of the insulin receptor, required for post-receptor insulin sensitivity and subsequent insulin-mediated glucose uptake, is dependent on adequate intracellular concentrations of magnesium [143]. As such, we also chose to investigate the relationship between serum magnesium and markers of IR; no significant association between them was observed. Inconsistencies have been present in previous studies examining this relationship, some supporting a negative correlation [233, 234] and others not [224]. One possible explanation for these differences is that serum magnesium may not accurately reflect intracellular magnesium levels, which may be low, even when serum levels are within the normal range [154]. Additional studies are warranted to further clarify the relationship between serum magnesium and markers of IR.

Our study had certain limitations, many of which were due to its cross-sectional design. Firstly, our use of a FFQ to evaluate patterns of dietary intake raises the

possibility of recall bias by subjects. However, the Willett FFQ chosen for this study is one of the most commonly applied tool for the evaluation of dietary intake in epidemiologic population-based studies [227, 228]. In addition, dietary magnesium is highly correlated with other micronutrients and dietary components believed to affect insulin sensitivity, such as vegetables, fruits, potassium, calcium, and fiber. Thus, it is very difficult to separate their independent effects [147, 151]. In addition, to avoid over-adjustment, we opted not to control for every available nutrient in our analysis. We did not measure or account for magnesium supplementation regarding daily magnesium intake, which could have potentially reduced the strength of the inverse association found between magnesium intake and IR makers. Furthermore, the reliability of serum magnesium levels in recognizing total body magnesium deficiency is unclear. Although intracellular magnesium concentrations are believed to provide a more accurate estimation of magnesium status, they are not generally easily measured [233]. Finally, our study enrolled Caucasian Newfoundlanders, so our findings may not be applicable to those from other ethnicities [156, 235].

In summary, our cross-sectional study investigated the relationship between dietary magnesium intake and insulin resistance among 2295 Newfoundlanders and Labradoreans. To our knowledge this study is the most comprehensive of its kind having controlled for major confounding factors, most specifically being dual energy x-ray absorptiometry (DXA) determined body fat percentage. Our findings suggest that higher dietary magnesium intake is associated with improved insulin sensitivity and this effect is

particularly beneficial for overweight and obese individuals in the general population along with pre-menopausal women. We also provide the first evidence that the association between dietary magnesium and insulin resistance is more strongly associated with %BF than BMI and the concomitant increase in %BF over BMI. Due to the fact that %BF more accurately represents adiposity than BMI, caution should be taken when attempting to utilize BMI as a measure of adiposity. Further large-scale prospective studies, where body fat is adequately accounted for and which enroll various ethnic groups, are needed to further elucidate the role of dietary magnesium in improving insulin function and preventing diabetes.

6

Chapter 6: Concordance of BAI and BMI with DXA in the Newfoundland Population

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6.1 - INTRODUCTION

With the rapid increase of obesity prevalence worldwide, attempts to develop a simple and low cost method to estimate adiposity more accurately than body mass index (BMI) are being made [170-172]. The body mass index [(Weight-kg)/(height-m)^2] was designed over 100 years ago [166] and due to its low cost and availability is the most commonly accepted method for estimating human adiposity by health professionals and the general population world-wide. In addition, BMI is currently the method utilized for the World Health Organization's (WHO) annual reports regarding obesity. However, although BMI is the most cost effective method to evaluate adiposity, it has been shown to have significant weaknesses in accurately determining adiposity. One such weakness of the BMI is that there are no sex specific criteria even though gender differences for body fat percentage have been well documented[167-169]. BMI also does not reflect neither the clear accumulation of body fat with age nor the inter-individual difference of body fat percentage among people within the same BMI category [15]. According to dual energy x-ray absorptiometry (DXA) body fat percentage measurements, BMI scores can mis-classify by one or even two obesity categories [14, 15]. Lastly, due to the fact that BMI was originally developed in Caucasian populations, the accuracy within various ethnic populations is problematic [176].

A study by Bergman et al. [170] recently proposed a new method to determine body fat percentage which has been named the body adiposity index (BAI). The BAI is calculated from two anthropometric measurements, height and hip circumference measurements were measured in meters (m) and centimeters (cm) respectively. The BAI

equation [BAI = Hip circumference (cm) / height(m)$^{1.5}$ – 18] was derived from the concordance between the body fat percentage of an Mexican-American population with resultants from proposed formulas utilizing various anthropometric measurements. Bergman et al. claim that their BAI equation can estimate percent body fat more reliably than BMI. However, like the BMI equation, it is unknown if BAI reflects the gender differences of both height and hip circumference measurements [180, 181] along with various dynamic anatomical measurements utilized to evaluate adiposity. Our laboratory and others have shown that equations such as BMI, which ignore gender differences for body fat percentage and the accumulation of body fat with age will, inaccurately estimate relative body fat and adiposity status [15, 167, 186, 236]. In addition to gender, obesity status can be a critical factor affecting the accuracy of methods that depend on bone structure measurements because there is little change of the skeletal measurement with changes in body weight or adiposity. At present there is very little data available regarding the influence of these two important factors on the performance of BAI.

Although, Bergman et al.[170] presuppose that BAI could be a better adiposity status measure than BMI, few studies to date have attempted to validate the BAI equation on a Caucasian population. Barreira et al.[182] letter to the editor was the first published material regarding the validation of the BAI equation on a Caucasian population against relative body fat. They found that the correlation between body fat percentage with BAI was very similar to that with BMI ($r = 0.82$ and $r = 0.83$ respectively) in women. However, the correlation of body fat percentage with BAI was not as similar BMI ($r = 0.75$ and $r = 0.81$) for men. Barreira et al. conclude that BAI equation was an effective

method for predicting body fat percentage for a Caucasian population, although further studies are required to support these findings. Therefore the objectives of the present study were to further investigate the performance of BAI according to sexes and obesity statuses (defined by body fat percentage) in our Caucasian population.

6.2 - SUBJECTS & METHODS

A total of 2660 subjects were recruited from an ongoing large-scale nutritional genetics study of human complex diseases called the CODING study [15, 16]. As BMI and %BF criteria are designed for individuals ≥ 20 yr, we excluded all participants below this age leaving us with a cohort of 2601 (1939 females, 663 men). Each individual completed a screening questionnaire that included information regarding physical characteristics, dietary habits, and physical activity levels. Dietary information was obtained from each participant completing the Willett Food Frequency Questionnaire (FFQ), which is a semi-quantitative method for the assessment of dietary intake patterns. The Willett FFQ is the most widely used dietary intake questionnaire for the study of nutritional information at the population level [237]. Physical activity was measured using the ARIC-Baecke Questionnaire, which consists of a Work Index, Sports Index, and Leisure Time Activity Index [226]. The primary method of subject recruitment was the use of posters and handouts. This literature was distributed throughout public facilities (offices, hospitals, and gyms) in the city of St. John's, Newfoundland. Inclusion criteria in the present study were as follows: (i) greater than 19 years age (ii) at least

third-generation Newfoundlander; (iii) healthy, without any serious metabolic, cardiovascular, or endocrine disease. This study was approved by The Human Investigations Committee for the Faculty of Medicine, Memorial University of Newfoundland and Labrador, St John's, Canada. All subjects provided written informed consent. Anthropometrics, body composition, and biochemical measurements were collected following a 12 hour fasting period.

Measurements of BAI, BMI and %BF

Subjects were weighed to the nearest 0.1 kg in standardized clothing as previously described by us (Health O Meter, Bridgeview, IL) [15-17]. Height was measured using a fixed stadiometer (nearest 0.1 cm). Hip circumference was measured as the largest circumference between the waist and thighs. Waist circumference was measured as the horizontal distance around the abdomen at the level of the umbilicus. Hip and waist measurements were recorded to the nearest 0.1 cm using a flexible metric measuring tape while the participant was in a standing position. BAI was calculated based on the equation reported in the Bergman paper [170]. BMI was calculated as weight in kilograms divided by participants height in meters squared. Whole body composition measurements including fat mass, lean body mass, and bone mineral densities were measured using DXA Lunar Prodigy (GE Medical Systems, Madison, WI). DXA can produce an accurate measurement of adipose tissue within the body with a low margin of error. For this reason, DXA is considered to be one of the most accurate measurements of adiposity and is commonly used as a standard compared to less accurate field methods

such as BMI. DXA measurements were performed on subjects following the removal of all metal accessories, while lying in a supine position as previously described by us [15-17]. Body fat percentage is determined as a ratio of fat mass over total body mass (including bone mineral densities) through the manufacturer's DXA software. Quality assurance was performed on our DXA scanner daily and the typical CV was 1.3% during the study period.

Statistical analysis

All data are reported as mean ± sd. The gender differences of variables measured were determined by an independent *t* test. Pearson correlation analysis was performed to compare the concordance between both BAI and BMI with body fat percentage (%BF) measured by DXA taking in consideration both gender and adiposity status. Adiposity status (normal weight, overweight, or obese) was determined by the Bray criteria (age and gender specific) [3] according to body fat percentage (%BF) measured DXA. The Lin's concordance correlation method was applied for both BAI and BMI with %BF, although the correlation coefficients were very poor for both. The Lin's concordance correlation coefficient represents the strength-of-agreement between two continuous variables, which if is lower than 0.90 is considered to be poor. Both of the BAI vs. %BF and BMI vs. %BF concordance correlation coefficients were much lower than the lowest acceptable coefficient, Pearson correlation analysis was used as the main validation method in this study. SPSS version 17.0 (SPSS, Chicago, IL) was used for all analyses.

Statistical analyses were two-sided and a *P* value <0.05 was to be statistically significant.

6.3 - RESULTS

Body composition characteristics of subjects in the study are shown in **Table 6.1**. Women on average were 3.7 years older than men. As most population studies have shown, women had lower body weight, height, BMI and waist circumference but higher %BF and hip circumference than men. DXA measurements on body composition revealed an average of 12.8% higher percent body fat in women than men. Interestingly, BAI estimation only demonstrates an average of 6.4% difference between women and men. Pearson correlations of both BAI and BMI with adiposity measurements within the entire study and each gender are shown in **Table 6.2.** In the entire cohort, the correlation coefficient between BMI and %BF (r = 0.56) was found to be lower than the correlation coefficient between BAI and %BF (r = 0.78). When analyses were performed according to gender the Pearson correlation coefficients of BAI with %BF were slightly lower than those of BMI with %BF. Pearson correlations of both BAI and BMI with adiposity measurements among normal-weight, overweight, and obese groups within the entire study and each gender are shown in **Table 6.3.** Normal-weight or overweight men and women had BAI vs. %BF correlation coefficients higher than the BMI vs. %BF correlation coefficients. However, BMI vs. %BF performed better than BAI vs. %BF in obese males and females.

Table 6.1 Composition characteristics of subjects.

	Entire Cohort (n = 2601)			Male (n = 662)			Female (n = 1939)			
	Mean	±	SD	Mean	±	SD	Mean	±	SD	P
Age [3]	42.34	±	13.05	39.55	±	14.4	43.30	±	12.4	0.000
Weight (kg) [2]	73.28	±	15.53	84.70	±	14.3	69.38	±	13.9	0.000
Height (cm) [2]	165.98	±	8.65	176.37	±	6.6	162.43	±	6.0	0.000
Waist (cm) [2]	91.35	±	14.27	96.09	±	13.3	89.73	±	14.2	0.000
Hip (cm) [3]	100.34	±	11.65	99.14	±	9.9	100.74	±	12.2	0.002
BMI (kg/m^2) [2]	26.55	±	4.99	27.23	±	4.3	26.31	±	5.2	0.000
BAI [3]	29.16	±	6.62	24.41	±	4.6	30.78	±	6.4	0.000
Body Fat (%) [3]	34.06	±	9.56	24.52	±	7.9	37.32	±	7.7	0.000

[1] All values are means ± SDs. Gender differences were analyzed by an independent t-test.

[2] Variable significantly greater in men

[3] Variable significantly greater in women

[4] Significance level for t-tests were set to $p \leq 0.05$

Table. 6.2 Pearson correlation between both BAI and BMI with body composition measurements within the entire cohort[1]

	Entire Cohort				Male				Female			
	BMI		BAI		BMI		BAI		BMI		BAI	
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.19	0.00	0.37	0.00	0.25	0.00	0.42	0.00	0.19	0.00	0.34	0.00
Weight (kg)	0.86	0.00	0.35	0.00	0.89	0.00	0.55	0.00	0.93	0.00	0.68	0.00
Height (cm)	-0.04	NS	-0.55	0.00	-0.11	0.00	-0.41	0.00	-0.14	0.00	-0.39	0.00
Waist (cm)	0.83	0.00	0.62	0.00	0.81	0.00	0.75	0.00	0.84	0.00	0.80	0.00
Hip (cm)	0.83	0.00	0.84	0.00	0.79	0.00	0.86	0.00	0.85	0.00	0.90	0.00
Body Fat (%)	0.56	0.00	0.78	0.00	0.70	0.00	0.67	0.00	0.76	0.00	0.74	0.00

[1] Pearson correlation analysis was used to determine the relationships between both BAI and BMI with body composition measurements.

[2] Significance levels were set to $p \leq 0.05$

Table. 6.3 Pearson correlation between both BAI and BMI with body composition measurements among NW, OW, OB men and women[1]

	Entire Cohort				Male				Female			
	Normal Weight (n= 849)				Normal Weight (n = 244)				Normal Weight (n = 605)			
	BMI		BAI		BMI		BAI		BMI		BAI	
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.19	0.00	0.50	0.00	0.34	0.00	0.49	0.00	0.22	0.00	0.49	0.00
Weight (kg)	0.78	0.00	-0.17	0.00	0.81	0.00	0.25	0.00	0.82	0.00	0.26	0.00
Height (cm)	0.15	0.00	-0.63	0.00	-0.13	0.05	-0.45	0.00	-0.15	0.00	-0.49	0.00
Waist (cm)	0.70	0.00	0.28	0.00	0.72	0.00	0.61	0.00	0.63	0.00	0.54	0.00
Hip (cm)	0.57	0.00	0.65	0.00	0.55	0.00	0.76	0.00	0.59	0.00	0.81	0.00
Body Fat (%)	0.05	NS	0.65	0.00	0.46	0.00	0.51	0.00	0.51	0.00	0.51	0.00
	Overweight (n = 776)				Overweight (n = 177)				Overweight (n = 599)			
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.25	0.00	0.41	0.00	0.18	0.01	0.50	0.00	0.29	0.00	0.47	0.00
Weight (kg)	0.78	0.00	-0.15	0.00	0.81	0.00	0.16	0.04	0.82	0.00	0.29	0.00
Height (cm)	0.08	0.02	-0.67	0.00	-0.10	NS	-0.46	0.00	-0.19	0.00	-0.55	0.00
Waist (cm)	0.70	0.00	0.32	0.00	0.56	0.00	0.60	0.00	0.69	0.00	0.62	0.00
Hip (cm)	0.59	0.00	0.69	0.00	0.47	0.00	0.77	0.00	0.65	0.00	0.80	0.00
Body Fat (%)	-0.09	0.01	0.62	0.00	0.18	0.02	0.42	0.00	0.43	0.00	0.49	0.00
	Obese (n = 976)				Obese (n = 241)				Obese (n = 735)			
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.10	0.00	0.29	0.00	0.15	0.02	0.34	0.00	0.08	0.03	0.22	0.00
Weight (kg)	0.80	0.00	0.20	0.00	0.86	0.00	0.43	0.00	0.89	0.00	0.54	0.00
Height (cm)	-0.08	0.01	-0.62	0.00	-0.10	NS	-0.49	0.00	-0.10	0.01	-0.41	0.00
Waist (cm)	0.73	0.00	0.49	0.00	0.71	0.00	0.61	0.00	0.76	0.00	0.72	0.00
Hip (cm)	0.79	0.00	0.80	0.00	0.79	0.00	0.81	0.00	0.79	0.00	0.85	0.00
Body Fat (%)	0.34	0.00	0.68	0.00	0.51	0.00	0.40	0.00	0.58	0.00	0.54	0.00

[1] Pearson correlation analysis was used to determine the relationships between both BAI and BMI with body composition measurements. NW, Normal-weight; OW, Overweight; OB, Obese. Subjects were classified on the basis of percentage body fat as Normal-weight, overweight, or obese according to criteria recommended by Bray

[2] Significance levels were set to $p \leq 0.05$

6.4 - DISCUSSION

To date, few studies have attempted to validate the Body Adiposity Index within a Caucasian population[182, 238]. To our knowledge this is the first study to attempt to validate the accuracy of the Body Adiposity Index against DXA measured percentage body fat among normal-weight, overweight, and obese Caucasian men and women. Simple adiposity assessment methods, such as BMI, are usually used to either evaluate obesity status at an individual level in clinics and health clubs or to estimate the adiposity of a sample population within a research investigation. In the entire cohort of this study, in which male and female subjects of all ages were mixed, the correlation coefficient between BAI and %BF was indeed much higher than that between BMI and %BF. This is generally consistent with the finding obtained in the Mexican-American and African-American populations, but the correlation coefficient between BAI and %BF in our CODING study was not as high as that shown in these two populations [170]. This result is more than likely due to the differences among the physical characteristics of these populations. The data from our large Caucasian population demonstrates that the BAI method is more strongly associated with DXA (the current gold standard for adiposity measurement) body fat percentage than BMI.

Although our data showed that the BAI performs better in estimating adiposity compared with BMI in a large population based study (men and women combined), what is of concern is that both of the BAI equation variables (hip circumference and height measurements) are essentially bone structure dependent. In addition, data from the adult population of our CODING study shows that women are generally shorter in height, have

smaller waist and larger hip circumferences than men. Therefore, longitudinal studies on measurement sensitive to the changes in adiposity similar for both men and women are warranted to aid in the development of an equation to accurately estimate adiposity. Our measurements of percent body fat in the CODING study indicate a large gender difference of adiposity. In the CODING study, women on average had 12.8% more total body fat than men. Due to the findings from our population studies we believe it is also important to further evaluate the efficiency of the BAI to predict %BF in women and men separately.

To have an equation that can accurately estimate adiposity for both men and women in the general population would be ideal due to the inherent gender differences. However, considering that hip circumference, the primary measure in the BAI equation, is larger in females than in men [15] it could potentially reflect gender differences to some degree. Conversely BAI adiposity scores are lower for men than women, and more closely represent the body fat percentage. Although the estimation of gender difference from the BAI measure is about half of the real range measured by DXA, this ability of BAI to properly reflect the gender differences in adiposity is certainly an advantage over BMI.

Finally, we were also interested in investigating the potential influence of adiposity on the accuracy of BAI and the comparison with BMI through DXA as a gold criterion. Therefore, the question that we wished to address was whether or not the two

skeletal system dependent variables would correctly reflect adiposity within various obesity statuses and if gender could be an important factor under these conditions. We found that the BAI method performed better than BMI among normal weight, overweight and obese groups when both women and men were combined. However, when women and men were analyzed separately the BAI only remained more strongly associated for the normal and overweight subjects. The BAI vs. %BF correlation coefficients for obese men and women were less than that of those for the BMI vs. %BF. Our findings indicate that caution should be taken when BAI is used to measure adiposity when obese women and men are separately evaluated. This finding indicates the weakness of BAI as a new evaluation method of adiposity in obese subjects. This weakness could be caused by the lack of measurement necessary to reflect adiposity and that the change in adiposity does not rely on height and hip circumference alone. The poor ability to evaluate adiposity in obese women and men is a problem that should be addressed in future study.

In summary, the present study attempted to validate the accuracy by which BAI estimates adiposity among over 2600 Caucasian men and women (from the CODING study) along with various adiposity statuses defined by percentage body fat measured by DXA. Firstly, BAI can reflect the gender difference in total body fat percentage between men and women. Secondly, our data indicates that the BAI method is a better estimate of adiposity than BMI in non-obese Caucasian subjects. Meaning, from our Caucasian population, body fat percentage evaluated by BAI correlates better than BMI in normal-weight and overweight men and women. BAI was less associated with percent body fat

than that of BMI in obese men and women. Therefore, caution should be taken when the BAI is used to evaluate adiposity in obese individuals. However, more studies evaluating the concordance of BAI with DXA body fat percentage among normal-weight, overweight and obese subjects are needed. We suggest that a measurement sensitive to the changes in adiposity for both men and women be incorporated into the present BAI equation to increase accuracy.

7

Chapter 7:Body Adiposity Index (BAI) versus Body Mass Index and Percent Body Fat as Correlates of Cardiometabolic Risk Factors in the Newfoundland population

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7.1 - INTRODUCTION

Obesity, characterized by excessive fat accumulation, is currently one of the top five leading risk factors for global mortality [18]. In Canada, the prevalence of obesity has tripled from 1985 to 2011, increasing from 6.1% to 18.3% [12]. The rise in obesity is mirrored by the rise of numerous obesity-related comorbidities including diabetes and cardiovascular disease [7-10]. This is especially relevant to the Canadian province of Newfoundland and Labrador, considering it has the highest rates of diabetes and obesity in Canada[12, 162]. The Canadian Diabetes Association has projected that by the year 2020 over 15% of Newfoundland and Labradorean's will be diabetic with an annual health care cost exceeding $360 million dollars[162]. However due to the excessive implementation of BMI, for obesity assessment, and its inability to differentiate fat mass from fat-free, the association of obesity with health risk factors has come into question [16, 239]. Air-displacement plethysmograohy (ADP), underwater weighing, magnetic resonance imaging (MRI) and/or dual-energy X-ray absorptiometry (DXA) are among the most precise measurements of body fat, conversely these methods are both expensive and impractical to assess obesity at the population level[163-165]. Therefore, recent focus has been placed on developing more accurate and cost effective methods of assessing adiposity for studying body fat associated disease risk [170, 188, 189].Due to its practicality and affordability the body mass index [(Weight (kg) / Height (m)^2] has become the most well-known and commonly used predictors of adiposity and was developed well over 100 years ago [166]. However a number of limitations, such as body fat content, body fat distribution, gender and age, have been recognized as weaknesses of

adiposity assessment for by BMI [14, 15, 167-169, 183-186]. Moreover, previously published studies from our laboratory have demonstrated that BMI significantly misclassifies obesity status and that risk for cardiometabolic disease revealed by %BF increases can be missed by inappropriately normal obesity status by BMI[15, 16, 207, 239].

Due to these limitations, the body adiposity index (BAI) was recently proposed as an alternative body composition estimate [170]. Unlike BMI, the BAI (hip circumference (cm)/[(height (cm)$^{1.5}$)-18]) was derived from %BF measured by DXA and does not involve the measurement of body weight. They reported that the BAI equation was more robust than BMI and required no statistical correction for gender or ethnicity [170]. However, regarding its prediction of %BF, it remains inconclusive whether BAI is a better predictor of %BF than BMI [170, 173-179]. Caution must be taken when interpreting the association of BAI with %BF as not all studies have employed a high resolution measure of body fat, such as DXA, which further adds to the controversial findings. Despite the current criticisms of the BAI equations to predict %BF, an equally important question is; Does BAI more closely represent the association of %BF with obesity-related CRFs than BMI.

To date, only a small number of studies have investigated the association of BAI and BMI with cardiometabolic metabolic risk factors (CRF)[190-194].Even fewer studies have included both insulin resistance and cardiovascular disease risk factors [190, 191].

Current studies have indicated that BAI is inferior to BMI as a predictor of CRFs. However, to the best of our knowledge, no large cross-section study has compared the association of insulin resistance and cardiovascular disease risk factors with BMI and BAI against their association with a high resolution measurement of body fat. Therefore, the objectives of the current study were to assess the relationship of CRFs with BAI, BMI and %BF and to also determine whether the association of BAI, or BMI, with CRFs was more concordant with the associated with %BF (measured by DXA) with CRFs in the Newfoundland Population.

7.2 - SUBJECTS & METHODS

Ethics Statement

Ethics approval for this study was received from the Human Investigation Committee, Faculty of Medicine, Memorial University, St. John's, Newfoundland, Canada. All subjects provided informed consent before taking part in this study.

Subjects

All of the 3059 subjects (852 men 2207 women) from this current study are volunteers of the general population of Newfoundland and Labrador and are participants in the ongoing cohort CODING (Complex Diseases in the Newfoundland Population: Environment and Genetics) study. Eligibility criteria inclusion criteria in the CODING study included: 1) at least a third generation Newfoundlander; 2) 19 years of

age or older; and 3) healthy, without any documented serious medical conditions. The primary method of subject recruitment for the CODING study was the use of posters and handouts. This literature was distributed throughout public facilities (offices, hospitals, and gyms) in the city of St. John's, Newfoundland and Labrador. All subjects completed screening questionnaires providing information about physical characteristics, physical activity, health status, and dietary practices. Anthropometrics, body composition, and biochemical measurements were collected following a 12 hour fast.

Anthropometric and Body Composition Measurements

Subjects were weighed (Health O Meter, Bridgeview, IL) to the nearest 0.1 kg in standardized clothing (hospital gown). Height was measured using a fixed stadiometer (nearest 0.1 cm). Hip circumference was measured as the largest circumference between the waist and thighs. Waist circumference was measured as the horizontal distance around the abdomen at the level of the umbilicus. Hip and waist measurements were recorded to the nearest 0.1 cm using a flexible metric measuring tape while the participant was in a standing position. Body mass index (BMI) was calculated by dividing the participant's weight in kilograms by their height in meters squared (kg/m^2). The body adiposity index (BAI) was calculated according to the equation [hip circumference (cm)/ $height(m)^{1.5}$– 18][170]. Percent body fat (%BF) was measured, in a supine position, utilizing dual-energy X-ray absorptiometry (DXA, Lunar Prodigy; GE Medical Systems, Madison, WI).DXA produces an accurate measurement of adipose

tissue within the body with a low margin of error. For this reason, DXA is considered to be one of the most accurate measurements of adiposity and is commonly used as a standard compared to less accurate field methods such as BMI. DXA measurements were performed on subjects following the removal of all metal accessories, while lying in a supine position [15-17]. Quality assurance was performed daily and the typical CV was 1.3% during the study period.

Biochemical measurements

Serum insulin concentrations were measured with the use of an immunoassay analyzer (Immulite; DPC, Los Angeles, CA).Insulin resistance and beta cell function were determined with the homeostasis model assessment (HOMA), as described by Matthews et al.[213].

HOMA-IR = [(Fasting Insulin (mU/L) x Fasting Glucose (mmol/L))/22.5]

HOMA-β = [(20x Fasting Insulin (mU/L))/(Fasting glucose (mmol/L) - 3.5)]

The serum concentrations of glucose, triacylglycerols (TG), high-density lipoprotein (HDLc) cholesterol and total cholesterol were measured by an Lx20 analyzer (Beckman Coulter Inc, Fullerton, CA) and the C16000 Architech Clinical Chemistry Analyzer (Abbott Diagnostics). Low-density lipoprotein (LDLc) cholesterol was calculated by the Friedewald equation (see below). The LDL cholesterol calculation is reliable in the absence of severe hyperlipidemia (TG > 4.6 mmol/L).

$$LDLc = [Total\_Cholesterol(mmol/L) - HDL\_Cholesterol(mmol/L) - (Triacylglycerol(mmol/L)/2.2)]$$

Statistical analysis

All data are reported as mean ± standard deviation (SD) unless otherwise stated. TG, insulin, HOMA-IR, and HOMA-β were log-transformed to normalize distributions for application of parametric statistical tests. Differences in biochemical and body composition measurements between men and women were assessed using one-way ANOVA. Pearson correlation analysis was performed to compare the relationship of BAI and BMI with CRFs to the relationship %BF with CRFs within the entire cohort and among men and women separately. BMI, BAI, and %BF were divided into tertiles (low, medium, or high) to study the relationship with cardiometabolic risk factors (Resting heart rate, systolic and diastolic blood pressure, total cholesterol, HDLc, LDLc, TG, glucose, insulin, HOMA-IR, and HOMA-β).The differences in CRFs among body composition tertiles were assessed using a one-way ANOVA. All statistical analyses were performed using PASW 19.0 (SPSS Inc., Chicago, IL). All tests were two-sided and a p value < 0.05 was considered to be statistically significant.

7.3 - RESULTS

Biochemical and body composition measurements for men, women, and the entire cohort

are presented in **Table 7.1**.

Anthropometric and Body Composition Measurements

Height, weight, BMI, waist circumference, and waist-to-hip ratio were all significantly greater for men compared to women. However, Age, %BF, and BAI were significantly greater in women than men (**Table 7.1**).

Cardiometabolic Risk Factors Measurements

TG, diastolic blood pressure, systolic blood pressure, glucose, insulin, HOMA-IR, and HOMA-β were significantly greater for men compared to women. However, total cholesterol, HDLc, and resting heart rate were significantly greater in women than men (**Table 7.1**).

Cardiometabolic Risk Factors according to BMI, BAI and %BF tertiles.

Cardiometabolic risk factors were assessed among tertiles (low, medium, or high) of BMI, BAI and %BF results within the entire cohort and presented in **Table 7.2**. The association of cholesterol, HDLc, LDLc, resting heart rate, glucose, and HOMA-β with BAI were concordant with their association with %BF (**Table 7.2**). The association of TG and insulin with BAI and BMI were concordant with their association with %BF. However, the association of HOMA-IR with BMI was concordant with its association with %BF (**Table 7.2**). Additionally, the association of systolic blood pressure with BAI and/or BMI were not concordant with their association with %BF or each other, and the

association of diastolic blood pressure with BAI was only concordant with its relationship to BMI (**Table 7.2**).

Cardiometabolic risk factors assessed among tertiles according to BMI, BAI and %BF among men and women are presented in **Table 7.3**. The association of HDLc and insulin with BAI were concordant with their association with %BF and the association of cholesterol with BMI was concordant with their association with %BF in men (**Table 7.3**). Additionally, the association of LDLc, TG, diastolic blood pressure with BMI and BAI were concordant with their associations with %BF in men. However, the association of systolic blood pressure, resting heart rate, glucose, HOMA-IR, and HOMA- β with BAI and/or BMI were not concordant with their associations with %BF in men (**Table 7.3**). Regarding women, the association of cholesterol, LDLc, glucose, insulin, HOMA-IR, and HOMA-β with BMI were similar to their association with %BF (**Table 7.3**). The association of HDLc, TG, diastolic blood pressure and systolic blood pressure with BMI and BAI are concordant with their associations with %BF in women. However, the association of resting heart rate with BAI and/or BMI was not associated with %BFin women (**Table 7.3**).

Pearson Correlation Analysis of Cardiometabolic Riskfactors with BMI, BAI and %BF

The Pearson correlation analyses of cardiometabolic risk factors with BMI, BAI

and %BF are presented in **Table 7.4**. The association of HDLc, TG, diastolic blood pressure, systolic blood pressure, glucose, insulin, HOMA-IR, and HOMA-β with BMI was stronger than their association with BAI and/or %BF**(Table 7.4)**. However, the association of cholesterol and LDLc with %BF and/or BAI were stronger than their association and BMI and the correlation coefficient of resting heart rate with %BF was stronger than BMI and BAI **(Table 7.4)**.Among men the correlation coefficient of TG, diastolic blood pressure, glucose, insulin, and HOMA-IR with BAI were greater than their association with BMI and/or %BF**(Table 7.4)**. Conversely, the association of HDLc, systolic blood pressure, and insulin with BMI were greater than their association with BAI and/or %BF and the association of cholesterol, LDLc, resting heart rate and HOMA-β with %BF were stronger than BMI and/or BAI **(Table 7.4)**.

Among women the Pearson correlation coefficient of HDLc, TG, diastolic blood pressure, systolic blood pressure, glucose, insulin, HOMA-IR and HOMA-β with BMI were greater than their association with BAI and/or %BF **(Table 7.4)**. Conversely, the Pearson correlation coefficient of cholesterol and LDLc with BMI were greater than their association of BAI and/or %BF and the association of resting heart rate with %BF was stronger than both BMI and/or BAI **(Table 7.4)**.

Table 7.1. Physical and Biochemical Characteristics[1,4]

	Entire Chort (n = 3059)		Male (n = 852)		Female (n = 2207)	
	Mean	SD	Mean	SD	Mean	SD
Age (y) [c]	42.82 ± 13.0		40.32 ± 14.0		43.78 ± 12.5	
Height (cm) [b]	166.44 ± 8.8		176.70 ± 6.5		162.48 ± 5.9	
Weight (kg) [b]	74.25 ± 16.4		86.50 ± 15.3		69.51 ± 14.1	
Waist (cm) [b]	92.21 ± 14.5		97.83 ± 13.5		90.04 ± 14.3	
Hip (cm)	100.55 ± 11.7		100.01 ± 10.0		100.76 ± 12.3	
Waist-Hip Ratio [b]	0.92 ± 0.1		0.98 ± 0.1		0.89 ± 0.1	
Body Mass Index (kg/m^2) [b]	26.71 ± 5.1		27.68 ± 4.5		26.34 ± 5.2	
Body Adiposity Index (%) [c]	29.06 ± 6.5		24.66 ± 4.6		30.75 ± 6.4	
Body Fat Percentage (%) [c]	33.96 ± 9.6		25.17 ± 8.2		37.35 ± 7.8	
Trunk Fat Percentage (%) [c]	36.25 ± 10.0		30.15 ± 9.7		38.61 ± 9.0	
Android Fat Percentage (%) [c]	41.33 ± 11.5		35.91 ± 11.5		43.42 ± 10.8	
Gynoid Fat Percentage (%) [c]	40.05 ± 10.1		28.39 ± 7.9		44.55 ± 6.7	
Total Cholesterol (mmol/L) [c]	5.10 ± 1.0		4.97 ± 1.1		5.15 ± 1.0	
HDLc (mmol/L) [c]	1.45 ± 0.4		1.21 ± 0.3		1.54 ± 0.4	
LDLc (mmol/L)	3.08 ± 0.9		3.10 ± 0.9		3.07 ± 0.9	
Triacylglycerols (mmol/L) [b]	1.20 ± 0.8		1.39 ± 1.0		1.13 ± 0.7	
Diastolic Blood Pressure (mmHg) [b]	80.81 ± 11.1		83.22 ± 10.9		79.89 ± 11.0	
Systolic Blood Pressure (mmHg) [b]	124.07 ± 16.5		131.49 ± 15.0		121.26 ± 16.1	
Resting Heart Rate (bpm) [c]	70.30 ± 11.9		66.61 ± 12.0		71.70 ± 11.6	
Glucose (mmol/L) [b]	5.12 ± 0.9		5.30 ± 1.0		5.04 ± 0.8	
Insulin (pmol/L) [b]	72.19 ± 66.9		78.00 ± 60.8		70.01 ± 68.9	
HOMA-IR [b]	2.50 ± 3.3		2.81 ± 3.4		2.38 ± 3.2	
HOMA-B [b]	134.89 ± 109.1		129.52 ± 104.1		136.91 ± 110.9	
HSCRP (mg/L) [c]	3.24 ± 4.4		2.53 ± 4.0		3.47 ± 4.5	

[a] All values are means ± SDs. Gender differences were analyzed by an independent t-test.

[b] Variable significantly greater in men

[c] Variable significantly greater in women

[d] Significance level for t-tests were set to $p < 0.05$

Table 7.2. Cardiometabolic Risk Factors of the Entire Cohort According to BMI, BAI, and %BF Tertiles.

	Entire Cohort		
	Low	**Medium**	**High**
BMI			
Total Cholesterol (mmol/L)	4.9 ± 1.0	5.2 ± 1.1[d]	5.3 ± 1.1[d]
HDLc (mmol/L)	1.6 ± 0.4	1.5 ± 0.3[d]	1.3 ± 0.3[d,e]
LDLc (mmol/L)	2.9 ± 0.9	3.1 ± 0.9[d]	3.2 ± 0.8[d]
Triacylglycerols (mmol/L)	0.9 ± 0.5	1.2 ± 0.7[d]	1.5 ± 0.9[d,e]
Diastolic Blood Pressure (mmHg)	77.0 ± 9.9	80.8 ± 10.2[d]	84.7 ± 11.6[d,e]
Systolic Blood Pressure (mmHg)	118.1 ± 14.5	124.5 ± 15.5[d]	129.7 ± 17.1[d,e]
Resting Heart Rate (bpm)	69.9 ± 11.8	69.5 ± 12.1	71.5 ± 11.7[d,e]
Glucose (mmol/L)	4.9 ± 0.5	5.1 ± 0.6[d]	5.4 ± 1.1[d,e]
Insulin (pmol/L)	50.3 ± 31.1	62.3 ± 36.8[d]	98.0 ± 93.9[d,e]
HOMA-IR	1.6 ± 1.1	2.1 ± 1.3[d]	3.6 ± 4.8[d,e]
HOMA-B	115.8 ± 85.7	128.6 ± 115.7	155.3 ± 115.9[d,e]
BAI			
Total Cholesterol (mmol/L)	4.8 ± 1.0	5.2 ± 0.9[d]	5.3 ± 1.1[d,e]
HDLc (mmol/L)	1.4 ± 0.4	1.5 ± 0.4[d]	1.4 ± 0.3[e]
LDLc (mmol/L)	2.9 ± 0.9	3.1 ± 0.8[d]	3.3 ± 0.9[d,e]
Triacylglycerols (mmol/L)	1.0 ± 0.6	1.2 ± 0.7[d]	1.4 ± 0.8[d,e]
Diastolic Blood Pressure (mmHg)	78.4 ± 9.9	80.8 ± 11.1[d]	83.3 ± 11.5[d,e]
Systolic Blood Pressure (mmHg)	122.2 ± 15.1	123.3 ± 16.7	126.8 ± 17.2[d,e]
Resting Heart Rate (bpm)	67.4 ± 12.1	70.6 ± 10.9[d]	72.9 ± 11.9[d,e]
Glucose (mmol/L)	5.0 ± 0.6	5.0 ± 0.6	5.3 ± 1.1[d,e]
Insulin (pmol/L)	55.5 ± 35.0	63.9 ± 42.9[d]	92.1 ± 92.8[d,e]
HOMA-IR	1.8 ± 1.3	2.1 ± 1.8	3.3 ± 4.7[d,e]
HOMA-B	112.3 ± 72.2	129.8 ± 104.7[d]	156.4 ± 130.1[d,e]
%BF			
Total Cholesterol (mmol/L)	4.8 ± 1.0	5.2 ± 1.1[d]	5.3 ± 1.1[d,e]
HDLc (mmol/L)	1.4 ± 0.4	1.5 ± 0.4[d]	1.4 ± 0.3[e]
LDLc (mmol/L)	2.9 ± 0.9	3.1 ± 0.8[d]	3.2 ± 0.9[d,e]
Triacylglycerols (mmol/L)	1.1 ± 0.8	1.2 ± 0.7[d]	1.3 ± 0.7[d,e]
Diastolic Blood Pressure (mmHg)	79.4 ± 10.6	80.5 ± 11.1	82.6 ± 11.2[d,e]
Systolic Blood Pressure (mmHg)	123.9 ± 15.9	123.1 ± 16.9	125.2 ± 16.6[e]
Resting Heart Rate (bpm)	66.5 ± 11.9	71.1 ± 11.2[d]	73.3 ± 11.6[d,e]
Glucose (mmol/L)	5.0 ± 0.7	5.1 ± 0.9	5.2 ± 0.9[d,e]
Insulin (pmol/L)	58.5 ± 38.0	69.9 ± 68.1[d]	85.0 ± 79.8[d,e]
HOMA-IR	2.0 ± 1.6	2.5 ± 3.9[d]	3.0 ± 3.4[d,e]
HOMA-B	113.8 ± 87.3	136.6 ± 119.3[d]	149.8 ± 112.1[d,e]

[a.] Data presented as mean ± SD. High-density lipoprotein cholesterol (HDLc). Low-density lipoprotein cholesterol (LDLc). Homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β).

[b.] Subjects were stratified into tertile (Low, Medium, High) based upon BMI, BAI, %BF.

[c.] Statistical Significance for one-way ANOVA were set to $p \leq 0.05$.

[d.] $p \leq 0.05$ versus low

[e.] $p \leq 0.05$ versus medium

Table 7.3. Cardiometabolic Risk Factors of Men and Women According to BMI, BAI, and %BF tertiles.

	Male			Female		
	Low	**Medium**	**High**	**Low**	**Medium**	**High**
BMI	(n = 284)	(n = 284)	(n = 284)	(n = 736)	(n = 736)	(n = 735)
Total Cholesterol (mmol/L)	4.5 ± 1.0	5.2 ± 1.1[d]	5.2 ± 1.1[d]	4.9 ± 0.9	5.2 ± 1.1[d]	5.3 ± 1.1[d]
HDLc (mmol/L)	1.3 ± 0.3	1.2 ± 0.3[d]	1.1 ± 0.2[d,e]	1.7 ± 0.4	1.6 ± 0.3[d]	1.4 ± 0.3[d,e]
LDLc (mmol/L)	2.8 ± 0.9	3.2 ± 0.9[d]	3.2 ± 0.9[d]	2.9 ± 0.8	3.2 ± 0.9[d]	3.2 ± 0.9[d]
Triacylglycerols (mmol/L)	0.9 ± 0.6	1.4 ± 0.9[d]	1.8 ± 1.1[d,e]	0.9 ± 0.4	1.1 ± 0.6[d]	1.4 ± 0.8[d,e]
Diastolic Blood Pressure (mmHg)	79.3 ± 9.9	83.3 ± 9.7[d]	87.1 ± 11.6[d,e]	76.0 ± 9.9	80.0 ± 10.3[d]	83.6 ± 11.4[d,e]
Systolic Blood Pressure (mmHg)	127.1 ± 13.4	131.2 ± 14.5[d]	136.3 ± 15.8[d,e]	115.8 ± 14.1	121.4 ± 15.2[d]	126.6 ± 17.1[d,e]
Resting Heart Rate (bpm)	65.1 ± 12.8	65.7 ± 11.8	69.0 ± 11.1[d,e]	70.4 ± 11.6	71.6 ± 11.3	73.1 ± 11.6[d,e]
Glucose (mmol/L)	5.0 ± 0.5	5.3 ± 0.9[d]	5.5 ± 1.2[d,e]	4.8 ± 0.5	5.0 ± 0.5[d]	5.4 ± 1.1[d,e]
Insulin (pmol/L)	54.7 ± 43.3	67.2 ± 42.5	104.1 ± 74.3[d,e]	47.7 ± 24.3	61.8 ± 35.6[d]	95.1 ± 100.4[d,e]
HOMA-IR	1.8 ± 1.6	2.3 ± 1.7	3.9 ± 4.8[d,e]	1.5 ± 0.9	2.0 ± 1.3[d]	3.4 ± 4.9[d,e]
HOMA-B	105.8 ± 84.9	115.6 ± 107.7	158.6 ± 106.8[d,e]	114.3 ± 74.5	136.4 ± 123.7[d]	155.3 ± 119.3[d,e]
BAI						
Total Cholesterol (mmol/L)	4.5 ± 0.9	5.1 ± 1.1[d]	5.3 ± 1.1[d,e]	4.9 ± 0.9	5.2 ± 0.9[d]	5.4 ± 1.1[d,e]
HDLc (mmol/L)	1.3 ± 0.3	1.2 ± 0.2	1.1 ± 0.2[d,e]	1.6 ± 0.4	1.6 ± 0.3[d]	1.4 ± 0.3[d,e]
LDLc (mmol/L)	2.8 ± 0.9	3.2 ± 0.9[d]	3.3 ± 0.9[d]	2.8 ± 0.8	3.1 ± 0.8[d]	3.3 ± 0.9[d,e]
Triacylglycerols (mmol/L)	0.9 ± 0.5	1.4 ± 0.9[d]	1.8 ± 1.1[d,e]	0.9 ± 0.4	1.1 ± 0.6[d]	1.4 ± 0.7[d,e]
Diastolic Blood Pressure (mmHg)	78.9 ± 9.4	82.9 ± 9.8[d]	88.1 ± 11.4[d,e]	76.9 ± 10.1	79.6 ± 10.5[d]	83.2 ± 11.4[d,e]
Systolic Blood Pressure (mmHg)	127.3 ± 13.0	131.1 ± 13.8[d]	136.2 ± 16.7[d,e]	116.3 ± 14.3	121.2 ± 15.6[d]	126.4 ± 16.8[d,e]
Resting Heart Rate (bpm)	64.4 ± 12.2	66.4 ± 11.6	69.2 ± 11.7[d,e]	70.5 ± 11.7	71.8 ± 10.9	72.9 ± 11.9[d]
Glucose (mmol/L)	5.0 ± 0.5	5.3 ± 0.7[d]	5.6 ± 1.3[d,e]	4.8 ± 0.5	5.0 ± 0.6[d]	5.3 ± 1.1[d,e]
Insulin (pmol/L)	53.5 ± 33.4	68.6 ± 44.1[d]	102.4 ± 77.1[d,e]	50.6 ± 27.0	60.2 ± 34.3[d]	93.6 ± 100.4[d,e]
HOMA-IR	1.7 ± 1.0	2.4 ± 1.6	3.9 ± 4.8[d,e]	1.6 ± 0.9	2.0 ± 1.3	3.4 ± 4.8[d,e]
HOMA-B	105.4 ± 80.5	112.2 ± 66.5	160.8 ± 133.7[d,e]	118.2 ± 69.9	129.4 ± 101.3	157.8 ± 137.9[d,e]
%BF						
Total Cholesterol (mmol/L)	4.5 ± 0.9	5.2 ± 1.1[d]	5.2 ± 1.1[d]	4.9 ± 0.9	5.3 ± 1.1[d]	5.3 ± 0.9[d]
HDLc (mmol/L)	1.3 ± 0.3	1.2 ± 0.2	1.1 ± 0.2[d,e]	1.7 ± 0.4	1.5 ± 0.3[d]	1.4 ± 0.3[d,e]
LDLc (mmol/L)	2.7 ± 0.8	3.3 ± 0.9[d]	3.3 ± 0.8[d]	2.8 ± 0.8	3.2 ± 0.9[d]	3.2 ± 0.8[d]
Triacylglycerols (mmol/L)	0.9 ± 0.6	1.5 ± 1.1[d]	1.7 ± 1.1[d,e]	0.9 ± 0.5	1.2 ± 0.7[d]	1.3 ± 0.7[d,e]
Diastolic Blood Pressure (mmHg)	78.4 ± 9.1	84.4 ± 10.1[d]	87.0 ± 11.6[d,e]	76.5 ± 10.2	80.4 ± 11.1[d]	82.8 ± 10.7[d,e]
Systolic Blood Pressure (mmHg)	127.3 ± 13.2	132.7 ± 14.6[d]	134.6 ± 16.1[d]	116.3 ± 14.5	122.6 ± 16.3[d]	124.9 ± 16.2[d,e]
Resting Heart Rate (bpm)	62.9 ± 11.7	67.1 ± 11.8[d]	69.9 ± 11.4[d,e]	69.1 ± 11.4	72.3 ± 11.1[d]	73.6 ± 11.8[d]
Glucose (mmol/L)	5.0 ± 0.5	5.4 ± 0.9[d]	5.5 ± 1.2d	4.8 ± 0.5	5.0 ± 0.7[d]	5.3 ± 0.9[d,e]
Insulin (pmol/L)	47.6 ± 30.7	71.9 ± 43.2[d]	103.7 ± 77.3[d,e]	52.6 ± 60.3	66.3 ± 73.3[d]	87.3 ± 66.8[d,e]
HOMA-IR	1.5 ± 0.9	2.6 ± 2.1[d]	3.9 ± 4.7[d,e]	1.7 ± 3.5	2.2 ± 3.6[d]	3.0 ± 2.3[d,e]
HOMA-B	93.2 ± 70.1	121.2 ± 110.9[d]	161.2 ± 106.7[d,e]	117.3 ± 79.8	136.9 ± 125.8[d]	152.3 ± 114.3[d,e]

[a.] Data presented as mean ± SD. High-density lipoprotein cholesterol (HDLc). Low-density lipoprotein cholesterol (LDLc). Homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β).

[b.] Subjects were stratified into tertile (low, Medium, High) based upon BMI, BAI,

[c.] Statistical significance for one-way ANOVA were set to $p < 0.05$.

[d.] $p < 0.05$ versus low

[e.] $p < 0.05$ versus medium

Table 7.4. Pearson Correlation analysis of BMI, BAI and %BF with Cardiometabolic Risk Factors.

	Entire Cohort						Male						Female					
	BMI		BAI		%BF		BMI		BAI		%BF		BMI		BAI		%BF	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Total Cholesterol (mmol/L)	0.07	≤ 0.001	0.10	≤ 0.001	0.13	≤ 0.001	0.14	≤ 0.001	0.17	≤ 0.001	0.22	≤ 0.001	0.04	≤ 0.001	0.08	≤ 0.001	0.09	≤ 0.001
HDLc (mmol/L)	-0.33	≤ 0.001	-0.27	≤ 0.001	-0.27	≤ 0.001	-0.32	≤ 0.001	-0.23	≤ 0.001	-0.28	≤ 0.001	-0.33	≤ 0.001	-0.27	≤ 0.001	-0.27	≤ 0.001
LDLc (mmol/L)	0.09	≤ 0.001	0.11	≤ 0.001	0.15	≤ 0.001	0.12	≤ 0.001	0.12	≤ 0.001	0.20	≤ 0.001	0.08	≤ 0.001	0.11	≤ 0.001	0.12	≤ 0.001
Triacylglycerols (mmol/L)	0.33	≤ 0.001	0.31	≤ 0.001	0.30	≤ 0.001	0.38	≤ 0.001	0.38	≤ 0.001	0.38	≤ 0.001	0.31	≤ 0.001	0.30	≤ 0.001	0.27	≤ 0.001
Diastolic Blood Pressure (mmHg)	0.29	≤ 0.001	0.13	≤ 0.001	0.10	≤ 0.001	0.30	≤ 0.001	0.30	≤ 0.001	0.27	≤ 0.001	0.26	≤ 0.001	0.21	≤ 0.001	0.22	≤ 0.001
Systolic Blood Pressure (mmHg)	0.28	≤ 0.001	0.06	≤ 0.001	0.03	NS	0.25	≤ 0.001	0.22	≤ 0.001	0.16	≤ 0.001	0.25	≤ 0.001	0.21	≤ 0.001	0.19	≤ 0.001
Resting Heart Rate (bpm)	0.11	≤ 0.001	0.24	≤ 0.001	0.30	≤ 0.001	0.18	≤ 0.001	0.26	≤ 0.001	0.28	≤ 0.001	0.13	≤ 0.001	0.15	≤ 0.001	0.21	≤ 0.001
Glucose (mmol/L)	0.27	≤ 0.001	0.23	≤ 0.001	0.17	≤ 0.001	0.18	≤ 0.001	0.16	≤ 0.001	0.10	≤ 0.001	0.30	≤ 0.001	0.26	≤ 0.001	0.20	≤ 0.001
Insulin (pmol/L)	0.50	≤ 0.001	0.37	≤ 0.001	0.29	≤ 0.001	0.48	≤ 0.001	0.47	≤ 0.001	0.46	≤ 0.001	0.50	≤ 0.001	0.46	≤ 0.001	0.39	≤ 0.001
HOMA-IR	0.52	≤ 0.001	0.36	≤ 0.001	0.26	≤ 0.001	0.48	≤ 0.001	0.46	≤ 0.001	0.45	≤ 0.001	0.52	≤ 0.001	0.47	≤ 0.001	0.39	≤ 0.001
HOMA-B	0.29	≤ 0.001	0.27	≤ 0.001	0.27	≤ 0.001	0.37	≤ 0.001	0.39	≤ 0.001	0.40	≤ 0.001	0.28	≤ 0.001	0.26	≤ 0.001	0.24	≤ 0.001

[a.] Body Mass Index (BMI), Body Adiposity Index (BAI), Body Fat Percentage (%BF), High-density lipoprotein cholesterol (HDLc). Low-density lipoprotein cholesterol (LDLc). Homeostasis model assessment of insulin resistance (HOMA-IR) and β-cell function (HOMA-β).

[b.] Pearson correlation analysis was used to assess the relationship of cardiometabolic risk factors with body composition measurements.

[c.] Significance levels were set to $p \leq 0.05$

7.4 - DISCUSSION

To date, few studies have explored the association of the body adiposity index (BAI), a new predictive equation for adiposity, with cardiometabolic risk factors (CRFs)[190-194]. To our knowledge this is the largest cross-sectional study to determine whether BAI, or BMI, is more closely associated with the relationship of adiposity (%BF measured by DXA) with cardiovascular disease and type-2 diabetes risk factors while taking in to consideration both gender and the severity of obesity. We found that BMI was more significantly associated with CRFs than either BAI or %BF in the Newfoundland population independent of sex. However, the association of CRFs with BAI was more strongly concordant with %BF than BMI in both the general population and when men and women were examined separately. Regarding the severity of adiposity, the influence of increasing BAI on CRFs was more strongly concordant with the increase in %BF than BMI. Conversely, sex stratified analysis revealed that the influence of increasing BMI on CRFs was more concordant with the increase of %BF than BAI in women. Although our results show that BMI has a stronger association with CRFs, the BAI more closely represents the association of CRFs with adiposity.

The association of CRFs with obesity are not well established. The majority of epidemiological studies evaluating the association of CRFs with obesity assume the BMI accurately predicts adiposity. The advent of new high resolution measurements of adiposity has allowed our laboratory [15-17, 178] and others [14, 15, 167-169, 183-

186]to have demonstrate that BMI is a very poor predictor of adiposity and obesity status. In attempts to overcome the poor concordance of BMI with %BF and the impractically of DXA to evaluate obesity at the population level, a study by Bergman et al. [170] recently described a new predictive equation for adiposity called the body adiposity index (BAI). The BAI is calculated from two anthropometric measurements, height and hip circumference measurements measured in meters (m) and centimeters (cm) respectively. The BAI equation [BAI = Hip circumference (cm) / height(m)$^{1.5}$ – 18] was derived from the concordance between the %BF measured by DXA anthropometric measurements in a Mexican-American population. A recent study from our large Caucasian population [178] found that BAI was a stronger predictor of DXA %BF than BMI. However, the predictive power of BAI is significantly attenuated when evaluating adiposity among men and women separately. Additionally, BAI was more strongly associated with DXA %BF than BMI among normal-weight, overweight and obese individuals, but the predictive power of BMI was stronger than BAI in obese men and women when evaluated within the sexes separately. Our findings[178], along with those of others[173, 175, 178, 179, 194, 197, 200, 201, 203, 204], clearly demonstrate that the sexual dimorphism of body fat distribution presents a challenge to the BAI equation in %BF. Few studies to date have investigated the association of BAI with cardiometabolic risk factors[190-192, 195-198], and even fewer have included both insulin resistance and cardiovascular disease risk factor measurements[190, 191]. More importantly, no large cross sectional study to date has thoroughly compared the associations of BAI and BMI

with CRFs, and against the association of DXA %BF with CRFs while taking into consideration potential effects of gender and adiposity.

The current study, and others [190-192, 195-198], demonstrate that BMI significantly over estimates the association of obesity with CRFs. Within the entire cohort or among men and women separately, BMI was more strongly associated CFRs than BAI or %BF. Only total cholesterol, LDLc and resting heart rate were more significantly associated with %BF than BMI. Since a number of studies have shown that body weight shows greater association with CRF than hip circumference independent of sex, it is not surprising that BMI, which includes body weight as one of its determinants, was found to be more strongly associated with CRFs than BAI. Studies by Snijder et al. [198] and Freedman et al. [199]showed that BMI and waist circumference more strongly correlated than BAI with LDLc, HDLc, TG, glucose, systolic blood pressure and diastolic blood pressure. While an investigation from the Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) study found that BMI was a better predictor of circulating lipids and glucose than BAI. BMI was more strongly associated with HDLc, TG, glucose, insulin, and HOMA-IR than BAI [192]. Another study reported that BMI was more significantly associated with glucose, systolic blood pressure and diastolic blood pressure[195]. A weight loss study on obese post-menopausal women by Elisha et al. [196] found that the reduction in BAI was not associated with changes in CRFs (total cholesterol, HDLc, TG, glucose, systolic blood pressure and diastolic blood pressure). Although the findings from these investigations

are similar to our own, none of these have evaluated which of BMI and BAI more closely associates with %BF. We found that BAIs relationship with CRFs more closely resembled that of %BF's with CRFs. These findings are supported by a recent study on 698 Mexican Americans, which showed that BMI was more strongly associated with CRFs than either BAI or %BF[191]. More importantly, although they did not show nor describe the data in detail, the authors state that the association of CRFs with BAI more closely resembled that of DXA %BF than BMI. Our findings would suggest that since strength of association of BMI with CRFs is greater than that of %BF, adiposity is not the only determinate of BMI's association with CRFs. Considering that BMI is a poor predictor of adiposity, and an even worse predictor of obesity status, it must be recognized that the association of BMI with CRFs is fairly independent of adiposity. This finding suggests that BAI maybe more closely representative of the association of CFRs with adiposity and should be explored further. Although, BMI has a stronger association with CRFs than does BAI, these findings should not lead to the claim that BMI is a more accurate predictor of adiposity associated CRFs than BAI.

We previously described the BAI as a stronger predictor of %BF in the general population [178]. The current study shows that BAI more closely resembles %BF in its relationship with CRFs. Considering this BAI could indeed be a useful tool in the study of obesity. The application of BAI in population based studies, where higher resolution assessments of adiposity are not available, could more accurately reflect the impact of obesity with CRFs, than BMI. However, our current study and others [173, 175, 178, 189, 194, 197, 200, 201] have clearly shown that BAI, like BMI, is not an effective

method for evaluating adiposity among men and women when sexes are examined separately. Due to the BAI not accounting for the sexual dimorphism of body fat distribution, caution must be exercised if BAI is to be used in male/female populations separately. Considering that obesity is growing rapidly across the globe, uncovering the association relative body fat with CRFs should also be investigated.

We were also interested in investigating the potential influence of adiposity on the association of CRFs and whether BMI or BAI would be more closely associated with the association of %BF (DXA as a gold standard criterion) with CRFs and if gender is an important factor. To account for adiposity we compared the association of increasing body fat percentage (tertile according to %BF measured by DXA) with CRFs against the association of increasing BMI and BAI on CRFs. The influence of increasing BAI on CRFs was more strongly concordant with that of increase in %BF than with BMI. However when the males and females were analyzed separately, the influence of increasing BMI on CRFs was more concordant with that of increase of %BF than BAI in women. Interestingly, the influence of increasing %BF on cardiovascular disease biomarkers was similar to the influence of increasing BAI and BMI, but the association of insulin resistance risk factors with increasing %BF were different from either BMI or BAI in men. This finding is not surprising. The BAI maybe a better predictor of CRFs for men due to this region being more sensitive to fat accumulation than women. This means that women can accumulate significantly more body fat in this region than men

before experiencing a significant increase in cardiovascular risk. Additionally, other studies have hypothesized that body fat may not reflect health risk in women as larger hip circumferences in overweight/obese women may provide some protection against accumulating CRFs and cardiovascular risk with obesity. If true, this could explain the stronger associations between BMI compared to BAI with the CRFs in the present study. Data from our laboratory supports this postulation having found that the accumulation of gynoid fat (body fat around the hips) is significantly more associated with CRFs in women than in men (data not shown). Also, waist circumference of men had stronger concordance with DXA %BF than that of the hip circumference measurement used in the BAI equation[178, 197].The weakness of BAI stems from height and hip circumferences not accurately estimating adiposity or its association with CRFs in both women and men equally. The association of BAI with CRFs is more closely %BF than BMI independent of sex. Therefore we believe that developing sex specific adiposity equation(s), to better predict %BF among men and women, would more accurately calculate adiposity and its association with CRFs. Having such an equation would allow for a higher resolution assessment of obesity in very large populations along with a more accurate influence of body fat accumulation on morbidity and mortality at the population level.

The primary limitation of the current study is that all the findings were derived only from Caucasian men and women. Validation of our findings by other studies using subjects of other ethnicities should be carried out before applying our conclusions to

other populations. Additionally, confounding variables such as: age, dietary intake of micro- and macronutrients, medications use, menopausal status, physical activity levels and smoking have not been accounted for. Although, the Pearson correlation analysis is adequate to support the findings in the present study, these aforementioned factors must be considered when attempting to further explore the physiological influence of adiposity on cardiometabolic risk factors. For example it is well known that both physical activity[240, 241] and dietary intake [153, 239] play a significant role development/attenuation of insulin resistance.

In summary, we investigated the associations of BAI and BMI with CRFs against the association of DXA %BF with CRFs while taking into consideration both gender and adiposity. The current study, which included 3059 subjects (852 men 2207 women) from the CODING study, revealed that BAI is more closely association with the relationship of %BF than BMI when subjects are not sub-categorized by gender. Our study also suggests, when considering adiposity, that the influence of increasing BAI on CRFs is similar to that of %BF in the general population. However, this association is significantly attenuated when assessing the influence of increasing BAI on CRFs amongst men and women examined separately. It is clear that BAI, like BMI, does not accommodated for the sexual dimorphism of body fat and significantly limits its ability to predict adiposity and its subsequent association with CRFs among men and women. We suggest that implementing measurements sensitive to the accumulation of body fat in men and women would aid in producing sex specific equations with greater predictive

power of adiposity and its association with CRFs. Our data suggests for the first time that BAI maybe a more meaningful predictor of adiposity associated CRFs than BMI in populations studies where separate examination of male and female data is not warranted.

8

Chapter 8:A New Sex Specific Predictive Equation for an Evaluationof Body Fat PercentageMore Accurate Than BMI and BAI

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8.1 - INTRODUCTION

The high prevalence of obesity and obesity-related comorbities have grown to epidemic proportions and are critically important global health issues [161]. Currently, 34.9% of the United States [4] and 18.3% of Canadians [12] are obese representing an annual health cost of ~$147 billion[5] and ~$7 billion [6], respectively. These fiscal burdens are principally attributable to the strong concordance of adiposity with serious chronic health conditions such as; type 2 diabetes, hypertension, cardiovascular disease, and various forms of cancer [6]. This is especially pertinent to the Canadian province of Newfoundland and Labrador considering it has the highest rates of obesity and diabetes in Canada [12]. Currently, 27.7% of Newfoundlanders and Labradorians are obese [12] and approximately 10% of the general population struggle with diabetes [162], which corresponds to an annual cost of $254 million dollars. Data from the Canadian Community Health Survey (CCHS) and the Canadian Diabetes Association (CDA) project that by the year 2020 over 71% on Newfoundland and Labradorians will be overweight/obese and the prevalence of diabetes will exceed 15%.

The body mass index (BMI), calculated as weight (kg) divided by height squared (m^2), is the most widely known and commonly used measure of obesity. In fact, the World Health Organization (WHO) defines obesity as the abnormal or excessive fat accumulation represented by a BMI value greater than or equal to 30 kg/m^2[18]. However, numerous studies have shown that BMI has many weaknesses and does not accurately predict fat accumulation in a substantial portion of the general population.

Specifically, BMI does not account for the well documented gender differences in weight, adiposity and body fat distribution [167-169]. Also, since does not BMI accurately distinguish fat mass from and fat-free mass[16], it often mis-classifies obesity status by up to two obesity categories compared with more accurate methods [14, 15]. More importantly, the false-negative classification of BMI defined obesity status reduces the identification of those at higher risk of cardiometabolic diseases with lower body fat content [187]. In fact, the WHO now admits that caution must be taken when utilizing BMI to classify obesity status[18].

In a recent attempt to address the limitations of BMI, and the impracticality of magnetic resonance imaging (MRI) and dual-energy x-ray absorptiometry (DXA) to measure percent body fat (%BF), Bergman et al. [170] developed an equation which could accurately predict %BF body fat percent measured by DXA. Regression analysis of anthropometric measurements with body fat produced the following formula for Body Adiposity Index (BAI), [hip circumference (cm)/ [(height $(m)^{1.5}$] – 18] [170]. However, like the BMI, it is unknown if BAI will reflect the gender differences of height and hip circumference measurements [180, 181] along with various dynamic anatomical measurements utilized to evaluate adiposity. Numerous studies to date, having evaluated the performance of BAI, have discovered that BAI is a strong predictor of percent body fat [173, 175, 178, 189, 194, 197, 200, 201]. However, these same studies also describe a significant reduction in the predictive power of BAI in evaluating adiposity when performed among men and women separately. The BAI tends to over-estimate, and underestimate DXA %BF among men and women, respectively [174, 189,201].

Additionally, the waist circumference of men had stronger concordance with DXA %BF than that of the hip circumference measurement used in the BAI equation [178, 197]. Recent, studies have also revealed that the predictive power of BAI significantly decreases among underweight and obese individuals [173, 174, 178,201-204]. Our previous findings [178], and those of others [173, 175, 178, 179, 194, 197, 200, 201, 203, 204], clearly demonstrate that not accounting for the sexual dimorphism of adiposity is the fundamental weakness of the BAI equation in predicting body fat percentage. This must be addressed to improve the development of future adiposity equations.

As the incidence of obesity and obesity-related diseases continues to increase, the need for an inexpensive and accurate assessment of adiposity has become of great importance to both clinical and epidemiological research. To date few sex specific adiposity equations, which accurately predict high resolution %BF measured by DXA, have been developed. Since BAI does not overcome the weakness of the BMI in predicting adiposity, the purpose of current study was to develop an equation(s) to more accurately predict body fat percentage among men and women in order to better define obesity status. An equation such as this would be of critical importance to study the biomarkers related to obesity development and obesity-related comorbidities at the population level.

8.2 - SUBJECTS & METHODS

Ethics Statement

This study was approved by The Health Research Ethics Authority (HREA) for

the Faculty of Medicine, Memorial University of Newfoundland and Labrador, St John's, Canada. All subjects provided written informed consent.

Subjects

A total of 3097 subjects (Male 881, Female 2254) were recruited from our ongoing large-scale Complex Diseases in the Newfoundland population: Environment and Genetics (CODING) study [15-17]. Anthropometric and body composition measurements were utilized to construct sex specific Body Fat Indices (BFI_m and BFI_f) to predict dual-energy x-ray absorptiometry (DXA). Sixty percent of entire cohort (male 518, female 1340) were randomly selected to construct the body fat percentage indices (BFI_m and BFI_f) and the remaining 40% (Male 363, Female 914) were implemented as a validation population. All participants of this current study were from the Canadian province of Newfoundland and Labrador. Inclusion criteria in the present study were as follows: (i) greater than 19 years age (ii) at least third-generation Newfoundlander; (iii) healthy, without any serious metabolic, cardiovascular, or endocrine disease. The primary method of subject recruitment involved the use of posters and handouts. This literature was distributed throughout public facilities (offices, hospitals, and gyms) in the city of St. John's, Newfoundland. Anthropometrics, body composition, and biochemical measurements were collected following a 12 hour fasting period.

Anthropometric and body composition measurements

Height (nearest 1.0 cm) and weight (nearest 0.1 kg) measurements were collected

and Body Mass Index (BMI) calculated. BMI was defined as weight divided by height squared (kg/m^2). Waist circumference (cm) was measured as the horizontal distance around the abdomen at the level of the umbilicus, and hip circumference (cm) was measured as the largest circumference between the waist and thighs. Height, waist and hip measurements were recorded to the nearest 0.1 cm. The body adiposity index (BAI) was calculated based on the equation reported in the Bergman paper [170]. Percent body fat (%BF) was measured utilizing a dual-energy X-ray absorptiometry (DXA, Lunar Prodigy; GE Medical Systems, Madison, WI). The current version of the enCORE software for the DXA data presented within this manuscript cannot differentiate visceral from subcutaneous fat. Therefore the %BF represents the summation of both subcutaneous and visceral fat. DXA produces an accurate measurement of adipose tissue within the body with a low margin of error. For this reason, DXA is considered to be one of the most reliable measurements of adiposity and is commonly used as a standard compared to less accurate field methods such as BMI. The enCORE (Ver 12.2, 2008, GE Medical Systems, Madison, WI) software package was used for DXA data acquisition. DXA measurements were performed on subjects following the removal of all metal accessories, while lying in a supine position in standardized clothing as previously described by us [15-17, 90]. Quality assurance was performed on the DXA scanner daily and the typical CV during the study period was 1.4%.

Statistical analysis

Creating Equation From Equation Dataset

All data are reported as mean ± SD. The gender differences of variables measured were determined by an independent *t* test. Pearson and partial (controlling for age) correlation analyses were performed to assess the association of anthropometric measurements with %BF measured by DXA. Due to the significant sex difference found of anthropometric measurements, and their association with %BF, the body fat index (BFI) was modeled for both males (BFI_m) and females (BFI_f) separately. This was done by determining the value of 'q' that maximizes the partial correlation (control age) between U / [Height(m)]q and %BF where 'U' is represented as one of the following anthropometric measurements; Waist, Hip, or Weight. Pearson's correlation, partial correlation (controlling for age), and Lin's concordance correlation were employed to compare the predictive power of BFI_m and BFI_f with %BF against that of BMI and BAI. The Lin's concordance correlation coefficient represents the strength-of-agreement between two continuous variables, which if it is lower than 0.90 is considered to be poor.

Validating Equation From Validation Dataset

Pearson's correlation, Lin's concordance and Lin's correlation coefficient of BFI (BFI_m ,BFI_f), BMI and BAI with percentage of body fat (%BF) measure by DXA from the equation and validation datasets were used to validate the BFI (BFI_m ,BFI_f) and compare the performance of each equation to predict %BF. Pearson's correlation, Lin's concordance and Lin's correlation coefficient of BFI (BFI_m ,BFI_f), BMI and BAI with percentage of body fat (%BF) measured by DXA among tertiles according to %BF in the validation dataset was used to compare the performance of each equation. Lastly, subjects

were stratified into tertiles (low, medium, high) based upon BFI, BMI, BAI, and %BF in the validation dataset to determine the correct classification counts/rates which determined by the number of subjects from each tertile group (BFI, BMI, and BAI), which corresponds to the tertile for %BF.R statistical software package version 2.15.2 (R development core team) was used for all analyses. Statistical analyses were two-sided and a P value ≤ 0.05 was considered to be statistically significant.

8.3 - RESULTS

Anthropometric and body composition

Anthropometric and body composition characteristics of the entire cohort, equation cohort, and validation cohort are shown in **Table 8.1**. Weight, height, waist circumference, waist-to-hip ratio and BMI were significantly greater in men than women in all three cohorts. Age, hip circumference, BAI, %BF, %TF, %AF, and %GF were significantly greater in women than men.

Pearson and Partial Correlations of Anthropometric Measurements With DXA %BF

Pearson correlation analysis revealed that age, weight and waist and hip circumference were positively associated with DXA %BF and that height and sex were negatively associated with DXA %BF (**Table 8.2**). Partial correlation analysis, controlling for age, demonstrated a very little overall effect on the significant association of anthropometric measurements with DXA %BF. However, partial correlation analysis,

controlling for age and sex increased the correlation of weight, and waist and hip circumference with DXA %BF, while diminishing its significant association with height.

Pearson and Partial Correlation of Anthropometric Measurements With DXA %BF Among Men and Women.

Pearson and partial (controlling age) correlation display that weight and waist and hip circumference are all positively associated with DXA %BF (**Table 8.3**). However, weight and hip circumference are more positively associated with DXA %BF in women but waist circumference correlates more positively with it in men. Moreover, the anthropometric measurement most positively associated with %BF in women was weight.

Partial Correlation of Anthropometric Measurements (Adjusted For Height) With DXA %BF Among Men and Women.

To determine the maximum partial correlation (controlling for age) of anthropometric measurements (waist, hip, and weight), while adjusting for height, with %BF we had to determine the value for the power coefficient 'q' for height. The value of 'q' that maximizes the partial correlation between the anthropometric measurements with %BF was q = 0.46415 (r = 0.73572) for [Waist / $(\text{Height})^q$], q = 0.49054 (r = 0.76197) for [Hip / $(\text{Height})^q$], and q = 1.70150 (r = 0.76375) for [Weight / $(\text{Height})^q$] (**Table 8.4**).Among men the value of 'q' to maximize the partial correlation between the anthropometric

measurements with %BF was q = 0.43530 (r = 0.78995) for [Waist / (Height)^{q}], q = 0.45750 (r = 0.75227) for [Hip / (Height)^{q}], and q = 1.68880 (r = 0.73880) for [Weight / (Height)^{q}] (**Table 8.4**). Among women the value of 'q' to maximize the partial correlation between the anthropometric measurements with %BF was q = 0.60588 (r = 0.71883) for [Waist / (Height)^{q}], q = 0.57666 (r = 0.77634) for [Hip / (Height)^{q}], and q = 1.81496 (r = 0.77699) for [Weight / (Height)^{q}]. For simplicity and efficiency of the new BFI, based on the analysis above, we choose Waist/Height$^{(q = 0.5)}$, Hip/Height$^{(q = 0.5)}$ and Weight/Height$^{(q = 2.0)}$.

The Association of BFI_m and BFI_f with DXA %BF

With the variables now in place Waist/Height$^{(q = 0.5)}$, Hip/Height$^{(q = 0.5)}$ and Weight/Height$^{(q = 2.0)}$ with the optimal weights and/or powers determined we now had to determine the coefficients for each variable and the constant which would maximize the Lin's concordance of the resultant of both the BFI_m and BFI_F equations with %BF measured by DXA. The Pearson's correlation coefficient (r) is a measure of precision and the bias correction factor (C_b) is a measure of accuracy. The product of r and C_b is the concordance correlation coefficient. The coefficients of 0.253819 for Waist/Height$^{(q = 0.5)}$, 0.3920474 for Hip/Height$^{(q = 0.5)}$ and 0.3541336 for Weight/Height$^{(q = 2.0)}$ along with a constant of 32.68815 resulted in the highest Lin's concordance (0.990683) and Lin's concordance coefficient (0.8155655) for the BFI_m with %BF in men (**Table 8.5**). The coefficients of 0.3695107 for Waist/Height$^{(q = 0.5)}$, 0.0683482 for Hip/Height$^{(q = 0.5)}$ and

0.5621411 for Weight/Height$^{(q = 2.0)}$ along with a constant of 11.45299 resulted in the highest Lin's concordance (0.9965971) and Lin's concordance coefficient (0.8047347) for the BFI_f with %BF in women (**Table 8.5**). Therefore the simplified sex specific predict equations for %BF are;

$$BFI \quad \begin{cases} 0.25\frac{\text{Hip(cm)}}{[\text{Height(m)}]^{1/2}} + 0.39\frac{\text{Waist(cm)}}{[\text{Height(m)}]^{1/2}} + 0.36\frac{\text{Weight(kg)}}{[\text{Height(m)}]^{2}} - 33 \\ 0.37\frac{\text{Hip(cm)}}{[\text{Height(m)}]^{1/2}} + 0.07\frac{\text{Waist(cm)}}{[\text{Height(m)}]^{1/2}} + 0.56\frac{\text{Weight(kg)}}{[\text{Height(m)}]^{2}} - 11 \end{cases}$$

Association of BFI (BFI_m ,BFI_f), BMI and BAI with percentage of body fat (%BF) in the equation and validation dataset.

The Pearson's correlation for BFI with %BF was significantly greater than the correlation for BMI and/or BAI with %BF within the entire cohort or among men and women separately in both the equation and validation dataset (**Table 8.6**). The Lin's concordance and Lin's concordance coefficient of BFI with %BF was also significantly greater than that for BMI and/or BAI with %BF within the entire cohort or among men and women separately in both the equation and validation dataset. In addition, the Pearson's correlation, Lin's concordance and Lin's concordance coefficient of BFI with %BF within the entire cohort or among men and women separately for the equation dataset was similar that on the validation dataset (**Table 8.6**).

Association of BFI (BFI_m ,BFI_f), BMI and BAI with percentage of body fat (%BF) among a tertiles according %BF in the validation dataset.

The Pearson's correlation, Lin's concordance and Lin's concordance coefficient of BFI with %BF was significantly greater than BMI and/or BAI with %BF at low, medium or high %BF (**Table 8.7**). The Pearson's correlation, Lin's concordance and Lin's concordance coefficient of BMI and/or BAI with %BF were inconsistent and in inaccurate among the tertiles according to %BF. This is especially prevalent for Lin's concordance and Lin's concordance coefficient of BMI and/or BAI with %BF in women in the medium and high %BF groups(**Table 8.7**).

The classification counts of tertiles of BFI (BFI_m BFI_f),BMI, and BAI according to tertiles of percent body fat (%BF) measured by DXA in the validation dataset.

The classification counts for the low, medium, and high BFI with the low, medium, and high %BF were significantly greater than either of the counts of BMI and/or BAI tertiles with the %BF tertiles within the entire cohort or among men and women separately in the validation dataset (**Table 8.8**). Therefore, the low, medium and high BFI tertiles groups each had the highest inclusion counts of subjects from the low, medium and high %BF groups.

The classification rates of tertiles of BFI (BFI_m BFI_f),BMI, and BAI according to tertiles of percent body fat (%BF) measured by DXA in the validation dataset.

The concordant classification of low, medium, and high BFI with the low, medium, and high %BF were significantly greater than either of the concordance for BMI and/or BAI tertiles with the %BF tertiles within the entire cohort or among men and women separately in the validation dataset. Therefore, the low, medium and high BFI tertiles groups each had the highest concordance of classification rates of subjects from the low, medium and high %BF groups. (**Table 8.9**)

Table 8.1 Anthropometric and body composition characteristics. [a,d]

CODING STUDY COHORT							
	Entire Cohort		Male		Female		
	n = 3097		n = 863		n = 2234		
	Mean	SD	Mean	SD	Mean	SD	p
Age (yr)[c]	42.53 ±	13.2	39.94 ±	14.1	43.53 ±	12.7	≤ 0.001
Weight (kg)[b]	74.22 ±	16.5	86.41 ±	15.6	69.51 ±	14.3	≤ 0.001
Height (m)[b]	1.66 ±	0.1	1.77 ±	0.1	1.62 ±	0.1	≤ 0.001
Waist (cm)[b]	92.09 ±	14.7	97.60 ±	13.8	89.95 ±	14.5	≤ 0.001
Hip (cm)	100.41 ±	12.0	99.80 ±	10.4	100.65 ±	12.6	NS
Waist-Hip Ratio [b]	0.92 ±	0.1	0.98 ±	0.1	0.89 ±	0.1	≤ 0.001
BMI (kg/m^2)[b]	26.71 ±	5.1	27.67 ±	4.6	26.34 ±	5.3	≤ 0.001
BAI (%)[c]	25.02 ±	7.1	23.66 ±	6.1	25.54 ±	7.4	≤ 0.001
BFI (%)[c]	28.69 ±	8.7	21.71 ±	6.6	31.39 ±	7.9	≤ 0.001
%BF (%)[c]	33.93 ±	9.7	25.14 ±	8.2	37.32 ±	7.9	≤ 0.001
EQUATION COHORT							
	Entire Cohort		Male		Female		
	n = 1858		n = 518		n = 1340		p
Age (yr)[c]	42.50 ±	13.1	40.37 ±	14.3	43.32 ±	12.4	≤ 0.001
Weight (kg)[b]	74.23 ±	16.5	86.33 ±	15.3	69.55 ±	14.5	≤ 0.001
Height (m)[b]	1.66 ±	0.1	1.77 ±	0.1	1.62 ±	0.1	≤ 0.001
Waist (cm)[b]	92.14 ±	14.9	97.77 ±	14.0	89.96 ±	14.6	≤ 0.001
Hip (cm)	100.54 ±	12.0	99.82 ±	10.2	100.82 ±	12.6	NS
Waist-Hip Ratio [b]	0.92 ±	0.1	0.98 ±	0.1	0.89 ±	0.1	≤ 0.001
BMI (kg/m^2)[b]	26.71 ±	5.1	27.62 ±	4.6	26.37 ±	5.3	≤ 0.001
BAI (%)[c]	25.04 ±	7.1	23.69 ±	6.2	25.56 ±	7.4	≤ 0.001
BFI (%)[c]	28.73 ±	8.8	21.66 ±	6.6	31.46 ±	8.0	≤ 0.001
%BF (%)[c]	33.97 ±	9.7	24.99 ±	8.4	37.44 ±	7.8	≤ 0.001
VALIDATION COHORT							
	Entire Cohort		Male		Female		
	n = 1239		n = 345		n = 894		
	Mean	SD	Mean	SD	Mean	SD	p
Age (yr)[c]	42.58 ±	13.4	39.29 ±	13.8	43.86 ±	13.1	≤ 0.001
Weight (kg)[b]	74.22 ±	16.5	86.54 ±	15.9	69.46 ±	14.0	≤ 0.001
Height (m)[b]	1.66 ±	0.1	1.76 ±	0.1	1.63 ±	0.1	≤ 0.001
Waist (cm)[b]	92.01 ±	14.5	97.36 ±	13.5	89.94 ±	14.3	≤ 0.001
Hip (cm)	100.22 ±	12.0	99.78 ±	10.7	100.40 ±	12.5	NS
Waist-Hip Ratio [b]	0.92 ±	0.1	0.97 ±	0.1	0.90 ±	0.1	≤ 0.001
BMI (kg/m^2)[b]	26.71 ±	5.1	27.76 ±	4.6	26.31 ±	5.2	≤ 0.001
BAI (%)[c]	24.99 ±	7.1	23.60 ±	5.8	25.52 ±	7.5	≤ 0.001
BFI (%)[c]	28.64 ±	8.6	21.78 ±	6.8	31.28 ±	7.8	≤ 0.001
%BF (%)[c]	33.87 ±	9.6	25.36 ±	8.0	37.15 ±	8.0	≤ 0.001

[a] All values are means ± SDs. Gender differences were analyzed by an independent t-test.

[b] Variable significantly greater in men.

[c] Variable significantly greater in women.

[d] Significance level for t-tests were set to $p < 0.05$.

Table 8.2 Correlation of anthropometrics with DXA %BF in the equation dataset.[d]

	Equation Cohort					
	Age	Sex	Weight	Height	Waist	Hip
Pearson r [a]	**0.252**	**-0.574**	**0.261**	**-0.452**	**0.459**	**0.648**
	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$
Partial r [b]		**-0.570**	**0.265**	**-0.421**	**0.426**	**0.626**
		$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$	$p \leq 0.001$
Partial r [c]			**0.722**	**-0.022**	**0.731**	**0.753**
			$p \leq 0.001$	NS	$p \leq 0.001$	$p \leq 0.001$

[a] Pearson correlation of variables with %BF.

[b] Partial correlation of variables with %BF controlling age.

[c] Partial correlation of variables with %BF controlling age and sex.

[d] Statistical significnace was set to $p \leq 0.05$.

Table 8.3 Correlation of anthropometrics with DXA %BF among men and women in the equtaion dataset.[c]

		Equation Cohort				
		Age	Weight	Height	Waist	Hip
Male	Pearson r [a]	**0.245**	**0.688**	**-0.044**	**0.799**	**0.757**
		$p \leq 0.001$	$p \leq 0.001$	NS	$p \leq 0.001$	$p \leq 0.001$
	Partial r [b]		**0.696**	**0.029**	**0.785**	**0.742**
			$p \leq 0.001$	NS	$p \leq 0.001$	$p \leq 0.001$
Female	Pearson r [a]	**0.234**	**0.730**	**-0.081**	**0.728**	**0.777**
		$p \leq 0.001$	$p \leq 0.001$	< 0.003	$p \leq 0.001$	$p \leq 0.001$
	Partial r [b]		**0.734**	**-0.045**	**0.711**	**0.764**
			$p \leq 0.001$	NS	$p \leq 0.001$	$p \leq 0.001$

[a] Pearson correlation of variables with %BF.

[b] Partial correlation of variables with %BF controlling age.

[c] Statistical significnace was set to $p \leq 0.05$.

Table 8.4 Partial correlation of anthropometric with DXA %BF controlling age in the equation dataset. [a,b,c]

Equation Cohort						
	[Waist / (Height)^q]		[Hip / (Height)^q]		[Weight / (Height)^q]	
	q	**r**	**q**	**r**	**q**	**r**
	0	0.73088	0	0.75260	0	0.72227
q.l	0.4	0.73562	0.4	0.76165	1.6	0.76359
Max.q	0.46415[a]	**0.73572**	0.49054[a]	**0.76197**	1.70150[a]	**0.76375**
q.u	0.5	0.73569	0.5	0.76197	1.8	0.76360
					2	0.76239
					1.5	0.76313
Male						
	[Waist / (Height)^q]		[Hip / (Height)^q]		[Weight / (Height)^q]	
	q	**r**	**q**	**r**	**q**	**r**
	0	0.78493	0	0.74198	0	0.69592
q.l	0.4	0.78991	0.4	0.75210	1.6	0.73866
Max.q	0.43530[a]	**0.78995**	0.45750[a]	**0.75227**	1.68880[a]	**0.73880**
q.u	0.5	0.78983	0.5	0.75218	1.7	0.73880
					1.8	0.73858
					1.9	0.73802
					2	0.73709
					1.5	0.73736
Female						
	[Waist / (Height)^q]		[Hip / (Height)^q]		[Weight / (Height)^q]	
	q	**r**	**q**	**r**	**q**	**r**
	0	0.71126	0	0.76420	0	0.73384
q.l	0.5	0.71860	0.5	0.77612	1.7	0.77681
Max.q	0.60588[a]	**0.71883**	0.57666[a]	**0.77634**	1.81496[a]	**0.77699**
q.u	0.7	0.71865	0.6	0.77632	1.9	0.77689
					2	0.77652
					1.6	0.77635
					1.5	0.77561

[a] Partial correlation of variables with %BF controlling age.

[b] q value such that partial correlation ' r ' is maximized.

[c] Statistical significnace was set to $p \leq 0.05$.

Table 8.5 The association of anthropometric measurements with %BF measured by DXA in the equation dataset.[e]

		[Waist / (Height)$^{0.5}$]	[Hip / (Height)$^{0.5}$]	[Weight / (Height)^{2}]	Maximum Concordance Coefficient [d]	Lin's Concordance Coefficient [c]	Pearson Correlation [a]	Lin's Concordance [b]
Male	BFI	0.253819	0.3920474	0.3541336	32.68815	**0.8155655**	0.8232356	**0.990683**
	BMI	0	0	1	0	0.5853212	0.7489622	0.7815096
	BAI	1	0	0	18	0.7306723	0.7741801	0.9438015
Female	BFI	0.3695107	0.0683482	0.5621411	11.45299	**0.8047347**	0.8074825	**0.9965971**
	BMI	0	0	1	0	0.3059767	0.7810922	0.3917293
	BAI	1	0	0	18	0.3240619	0.7222169	0.4487044

[a] Pearson's correlation (r) of BFI with %BF.

[b] Lin's condordance (C_b) of BFI with %BF.

[c] Lin's condordance coefficient [(C_b) x (r)] of BFI with %BF.

[d] Maximum concordance coefficient of BFI with %BF.

[e] Statistical significnace was set to $p < 0.05$.

Table 8.6 Pearson's correlation, Lin's concordance and Lin's correlation coefficient of BFI (BFI_m ,BFI_f), BMI and BAI with percentage of body fat (%BF) measure by DXA in the validation dataset. [d]

		EQUATION COHORT			VALIDATION COHORT		
		n = 1858 (Male 518, Female 1340)			n = 1277 (Male 363, Female 914)		
		Pearson Correlation [a]	Lin's Concordance [b]	Lin's Concordance Coefficient [c]	Pearson Correlation [a]	Lin's Concordance [b]	Lin's Concordance Coefficient [c]
Entire Cohort	BFI	**0.878**	**0.999**	**0.878**	**0.863**	**0.999**	**0.862**
	BMI	0.563	0.576	0.325	0.564	0.579	0.327
	BAI	0.662	0.616	0.408	0.650	0.616	0.400
Male	BFI	**0.807**	**0.994**	**0.803**	**0.797**	**0.988**	**0.788**
	BMI	0.781	0.392	0.306	0.778	0.400	0.312
	BAI	0.722	0.449	0.324	0.695	0.469	0.326
Female	BFI	**0.823**	**0.987**	**0.813**	**0.792**	**0.987**	**0.782**
	BMI	0.749	0.782	0.585	0.737	0.813	0.599
	BAI	0.774	0.944	0.731	0.753	0.923	0.695

[a] Pearson's correlation (r) of BFI with %BF.

[b] Lin's condordance (C_b) of BFI with %BF.

[c] Lin's condordance coefficient [(C_b) x (r)] of BFI with %BF.

[d] Statistical significnace was set to $p < 0.05$.

Table 8.7 Person's correlation, Lin's concordance and Lin's correlation coefficient of BFI (BFI_m ,BFI_f) , BMI and BAI with percentage of body fat (%BF) measured by DXA among tertiles according to %BF in validation dataset. [a,d]

		Entire Cohort (n = 1239)			Male (n = 345)			Female (n = 894)		
		%BF			%BF			%BF		
		Low	Medium	High	Low	Medium	High	Low	Medium	High
BFI	Pearson Correlation [b]	**0.664**	**0.371**	**0.604**	**0.567**	**0.416**	**0.415**	**0.522**	**0.467**	**0.514**
	Lin's Concordance [c]	**0.941**	**0.799**	**0.879**	**0.905**	**0.674**	**0.700**	**0.707**	**0.814**	**0.815**
	Lin's Concordance Coefficient [d]	**0.625**	**0.297**	**0.531**	**0.513**	**0.280**	**0.291**	**0.369**	**0.380**	**0.419**
BMI	Pearson Correlation [b]	0.207	0.035	0.581	0.447	0.402	0.350	0.423	0.444	0.567
	Lin's Concordance [c]	0.887	0.209	0.167	0.280	0.875	0.847	0.371	0.077	0.133
	Lin's Concordance Coefficient [d]	0.184	0.007	0.097	0.125	0.352	0.296	0.157	0.034	0.075
BAI	Pearson Correlation [b]	0.428	0.161	0.422	0.528	0.371	0.291	0.421	0.352	0.357
	Lin's Concordance [c]	0.823	0.185	0.219	0.825	0.645	0.508	0.378	0.111	0.186
	Lin's Concordance Coefficient [d]	0.352	0.030	0.093	0.436	0.239	0.148	0.159	0.039	0.066

[a] Subjects were stratified in a tertile (low, medium, high) based upon %BF measured by DXA in the validation dataset.

[b] Pearson's correlation (r) of BFI with %BF.

[c] Lin's condordance (C_b) of BFI with %BF.

[d] Lin's condordance coefficient [(C_b) x (r)] of BFI with %BF.

[e] Statistical significnace was set to $p < 0.05$.

Table 8.8 The classification counts of tertiles of BFI (BFIm BFIf),BMI, and BAI according to tertiles of percent body fat (%BF) measured by DXA in the validation dataset. [a]

		Entire Cohort (n = 1,239)			Male (n = 345)			Female (n = 894)		
		%BF			%BF			%BF		
		Low	Medium	High	Low	Medium	High	Low	Medium	High
		n = 413	n = 413	n = 413	n = 115	n = 115	n = 115	n = 298	n = 298	n = 298
BFI	Low	**333**	75	5	**89**	22	4	**233**	59	6
	Medium	79	**266**	68	23	**69**	23	62	**176**	60
	High	1	72	**340**	3	24	**88**	3	63	**232**
BMI	Low	225	169	19	81	32	2	225	71	2
	Medium	136	144	133	29	59	27	68	166	64
	High	52	100	261	5	24	86	5	61	232
BAI	Low	255	131	27	85	24	6	212	72	14
	Medium	138	173	102	26	65	24	79	140	79
	High	20	109	284	4	26	85	7	86	205

[a] Subjects were stratified in a tertile (low, medium, high) based upon BFI, BMI, BAI, and %BF in the validation dataset. The correct classifcations counts were determined by the number of subjects from each tertile of BFI, BMI, and BAI, which corresponds to the tertile for %BF.

Table 8.9 The classification rate of tertiles of BFI (BFIm BFIf),BMI, and BAI according to tertiles of percent body fat (%BF) measured by DXA in the validation dataset. [a]

		Entire Cohort (n = 1,239)			Male (n = 345)			Female (n = 894)		
		%BF			%BF			%BF		
		Low	Medium	High	Low	Medium	High	Low	Medium	High
		n = 413	n = 413	n = 413	n = 298	n = 298	n = 298	n = 115	n = 115	n = 115
BFI	Low	**0.806**	0.182	0.012	**0.774**	0.191	0.035	**0.782**	0.198	0.020
	Medium	0.191	**0.644**	0.165	0.200	**0.600**	0.200	0.208	**0.591**	0.201
	High	0.002	0.174	**0.823**	0.026	0.209	**0.765**	0.010	0.211	**0.779**
BMI	Low	0.545	0.409	0.046	0.704	0.278	0.017	0.755	0.238	0.007
	Medium	0.329	0.349	0.322	0.252	0.513	0.235	0.228	0.557	0.215
	High	0.126	0.242	0.632	0.044	0.209	0.748	0.017	0.205	0.779
BAI	Low	0.617	0.317	0.065	0.739	0.209	0.052	0.711	0.242	0.047
	Medium	0.334	0.419	0.247	0.226	0.565	0.209	0.265	0.470	0.265
	High	0.048	0.264	0.688	0.035	0.226	0.739	0.024	0.289	0.688

[a] Subjects were stratified in a tertile (low, medium, high) based upon BFI, BMI, BAI, and %BF in the validation dataset. The correct classifcations rates were determined by the ratio of subjects from each tertile of BFI, BMI, and BAI, which corresponds to the tertile for %BF.

8.4 - DISCUSSION

The current study introduces a unique sex specific equation that more accurately predicts relative body fat (%BF) measured by dual-energy x-ray absorptiometry (DXA). The BFI_m and BFI_f equations were derived from common clinical anthropometric measurements, taking into account dimorphic sex differences of adiposity, to more directly predict %BF then using the body mass index (BMI) or the body adiposity index (BAI). We have demonstrated that accounting for gender specific body fat distribution in our BFI equations have effectively increased the predictive power of %BF over that of both BAI and BMI in the general population and among men and women at various extremes of adiposity.

Our laboratory [15, 16, 178] and others [167-169, 186] have shown that adiposity assessments, which ignore the gender differences of body fat and body fat distribution, will inaccurately estimate relative body fat and obesity status. Since, BMI does not account for the well documented gender differences in weight, adiposity and body fat distribution [167-169], it would not be surprising that a sex specific equation would perform better than BMI at predicting adiposity. However, a new predictive equation called the body adiposity index has been developed and claims to effectively overcome gender differences without addressing them directly. We tested this association through a validation study of the BAI equation on the large Caucasian population found in our CODING study[178]. Within the study entire cohort, the correlation coefficient between

BAI and %BF was stronger than the association of BMI with %BF[178]. This has also found in the majority of other studies attempting to validate the BAI equation [173, 175, 178, 189, 194, 197, 200, 201]. However, these studies also demonstrated that the BAI was not a better predictor of DXA %BF compared with BMI when men and women were investigated separately. We concluded that a measurement sensitive to the sex specific changes in adiposity for both men and women separately must be used to increase the predictive power of BAI[178]. Considering our previous findings and the critical need for an equation to more accurately predict adiposity at the population level we decided to create sex specific equations to predict body fat percentage measured by DXA.

Many epidemiological studies, defining obesity by BMI, have claimed that a number of metabolic and cardiovascular risk factors are related to adipose tissue accumulation. However, since our laboratory and others have shown that BMI cannot accurately predict adiposity nor define obesity, it is possible that the majority of epidemiological obesity studies on large populations inaccurately define obesity and therefore its association on metabolic and cardiovascular disease. This fact is evident in a number large number of studies showing that body fat percentage is significantly higher among women than men having the same BMI values. This is also one of the strongest pieces of evidence demonstrating the sexual dimorphism of body fat. Not only does BMI fail to accurately predict adiposity associated risk factors, but it also does not consider sex-specific body fat distribution and its effect on adiposity associated health risk factors. It is imperative that a more accurate measurement of body fat in the field be developed to more accurately predict the effects of adiposity on various health risk factors in both men

and women. The goal of the present study was to develop sex specific equations to more accurately predict relative body fat (determined using DXA) than BMI.

The initial steps taken to create our sex specific equation was to assess the association of typical clinical anthropometric measurements such as weight (kg), height (m), hip and waist circumferences (cm) within the entire cohort and among men and women separately. Pearson correlation and partial correlation analysis (controlling for age) revealed that weight, waist circumference and hip circumference had reasonable associations with %BF, and partial correlation analysis controlling for gender revealed an amplified positive association of these variables with %BF. As was initially hypothesized the gender dimorphism of body fat has a significant influence on the association of anthropometric measurements with adiposity. This was further supported by the fact that the above anthropometric measurements were also more strongly associated with %BF among men and women separately. In addition, because height was significantly negatively associated with %BF and nullified due to the consideration of gender, height was also considered an important factor in the prediction of body fat. This was further supported by the observation that the correlations of weight, waist circumference and hip circumference with %BF were improved when correcting for height.

The next step involved determining the exponential weighting, that when applied to height, would optimize correction of height for each of the anthropometric measurements and maximizing their relationship with %BF. The following model was

employed to determine the exponential weighting. We setup that the exponential weighting value 'q' upon height which maximizes the correlation between the anthropometric measurements value 'U' and %BF [$\%BF = U / (height)^q$]. Among men the optimal 'q' for weight, waist circumference and hip circumference was 1.68880 ($r = 0.73880$, $p \leq 0.001$), 0.43530 ($r = 0.78995$, $p \leq 0.001$), and 0.45750 ($r = 0.75227$, $p \leq 0.001$) respectively. Among women the optimal 'q' for weight, waist circumference and hip circumference was 1.81496 ($r = 0.77699$, $p \leq 0.001$), 0.60588 ($r = 0.71883$, $p \leq 0.001$), and 0.57666 ($r = 0.77634$, $p \leq 0.001$) respectively. Due to minimal and the non-significant impact, 'q' values for both men and women were modified to 2.0 (Men - $r = 0.73709$, Women - $r = 0.77652$; $p \leq 0.001$) for weight, 0.5 (Men - $r = 0.78983$, Women - $r = 0.71860$; $p \leq 0.001$) for waist circumference, and 0.5 (Men - $r = 0.75218$, Women - $r = 0.77612$; $p \leq 0.001$) for hip circumference, to simplify the equation.

The final step in the design of the equation was to determine the optimal weight/coefficients that would be applied to each anthropometric measurement (appropriately corrected for height) and the resulting constant which would produce a body fat percentage maximally concordant with %BF measured by DXA. We set BFI (BFI_m and/or BFI_f) the sum of the product of the optimal coefficient (a_1, a_2, a_3) applied to each height corrected anthropometric measurement minus a corresponding optimal constant (C). The corresponding model was; [$BFI = a_1(hip/(height)^{0.5}) + a_2(waist/(height)^{0.5}) + a_3(Weight/(height)^{2.0}) - C$]. Among men the optimal coefficients were 0.3541336, 0.253819, and 0.3920474 for weight, waist circumference and hip

circumference respectively with a constant of 32.68815 resulting in an optimal Lin's concordance of 0.990683. Among women the optimal coefficients were 0.3695107, 0.0683282, and 0.5621411 for weight, waist circumference and hip circumference respectively with a constant of 11.45299 resulting in an optimal Lin's concordance of 0.9965971. Therefore, the resulting sex specific equations were;

$$BFI_m = 0.25[hip(cm)/Height(m)^{0.5}]+0.39[Waist(cm)/Height(m)^{0.5}]+0.36[Weight(kg)/Height(m)]^2 - 33$$

$$BFI_f = 0.37[hip(cm)/Height(m)^{0.5}]+0.07[Waist(cm)/Height(m)^{0.5}]+0.56[Weight(kg)/Height(m)]^2 - 11$$

With the sex specific equations now optimally design the final step was to validate the predictive power of these equations in the validation dataset. Our results demonstrated that the Pearson's correlation, Lin's concordance and Lin's correlation coefficient of our new BFI (BFI_m and BFI_f) with DXA %BF were greater than for BMI and/or BAI in the entire cohort and among men and women separately within the equation and validation dataset. Most noticeably the association of BFI with %BF remained remarkably constant when assessed among men and women separately. Although, both BAIs and BMIs association with %BF become inconsistent when assessed among sexes, this finding is not surprising. A number of studies to date have evaluated the performance of BAI and discovered it is as good or better predictor of %BF than BMI in the general population [173, 175, 178, 189, 194, 197, 200, 201]. However, these studies also discovered a significant reduction in the predictive power of BAI when evaluating adiposity among men and women separately. Generally, the BAI tends to

over-estimate, and underestimate DXA %BF among men and women, respectively[174, 189,201].

The current study demonstrates that our new BFI equation effectively incorporates the significant differences in body fat distribution between men and women. Although we have designed and validated equations which can better predict %BF than BMI and BAI in the general population and among men and women separately it is not clear if BFI would better predict body fat at low or high levels of DXA %BF. After stratifying subjects into tertiles (low, medium, high) according to %BF the Pearson correlation, Lin's concordance, and Lin's correlation coefficient of BFI with %BF were significantly greater than that of BMI and BAI with %BF at the low, medium and high %BF. This suggests that the BFI equations are better predictors of %BF at the variable extremes/ranges of adiposity. A number of studies have also shown that the predictive power of BAI and BMI significantly decreases among underweight and obese individuals [173, 174, 178,201-204]. Our current study also revealed a significant reduction in the predictive power of both BMI and BAI, especially in women.

Lastly, we explored whether the BFI equations will better classify %BF than BMI and BAI. Subjects were stratified into tertiles (low, medium, high) based upon BFI, BMI, BAI, and %BF in the validation dataset. The correct classifications counts were determined by the number of subjects from each tertile of BFI, BMI, and BAI, which corresponds to %BF tertiles. Our results revealed that the classification counts and/or rates of BFI with %BF were greater than for the BAI and BMI for each corresponding

%BF tertile (low, medium, and high).

Although our equations are more complicated than either BAI or BMI, the sex specific BFI equations designed in the current study more accurate predict %BF. Not only have we shown that accounting for the sexual dimorphism of body fat in our equation effectively increased the predictive power of adiposity, but it can also increase the predictive power of %BF among various extremes of adiposity. This is a very important point to consider due to the fact that the primary purpose of the work is based upon the assumption that adiposity is a critical determinant to chronic disease development at the population level. Meaning that if we are to further understand the influence of body fat on various health conditions, a method like BMI, which cannot predict relative body fat, must be replaced with a more accurate predictor of adiposity for population based studies. Therefore, due to rising prevalence of obesity across the globe a methodology, such as the equations we have designed, are necessary and significant contribution to assess adiposity and its subsequent association with disease.

Our study had certain limitations, many of which were due to its cross-sectional design. Firstly, the design and validation of the BFI_m and BFI_f we derived only from a Caucasian population. Therefore, before the BFI_M and BFI_f equations can be implemented globally large scale validation studies involving different or mixed ethnicities must be performed.

In summary, our study has introduced two new equations to directly predict the body fat percentage of men and women without the need of expensive equipment. The BFI_m and BFI_f, which accurately calculate %BF measured by DXA, were derived from the anthropometric measurements most strongly assorted with adiposity in men and women, respectively. Considering that we addressed the sexual dimorphism of body fat distribution, over looked by both BMI and BAI, the results indicate that our new sex specific equations better predict relative body fat. Additionally, having considered the sexual dimorphism of adiposity, we believe that our equations will be more sensitive to the change in %BF observed during weight gain/loss. The BFI_m and BFI_f equations will be a valuable tool for physicians, and epidemiological researchers, wishing to accurately assess relative body fat and its association with chronic diseases.

9

Chapter 9: Summary & Conclusions

9.1 – Summary & Conclusion

Obesity, defined by the excessive accumulation of adipose tissue, is one of the leading causes of death around the world. The high prevalence of obesity and obesity-related comorbities have grown to epidemic proportions and are critically important global health issues. This thesis has contributed to further our understanding of obesity by exploring: 1) the association of appetite regulating hormones with adiposity and obesity status; 2) the response of appetite and energy regulating hormones to a positive energy challenge; 3) the association of dietary magnesium intake with insulin resistance adjusted for critical confounding factors; 4) the validity of the body adiposity index (BAI) to predict adiposity in a Caucasian population; 5) the association of BAI with cardiometabolic risk factors (CRFs), and 6) the development of two new sex-specific equations to predict body fat measured by dual-energy x-ray absorptiometry (DXA) better than BMI or BAI. By implementing a multifaceted approach, we provide a strong comprehensive overview of the etiology of obesity.

In **Chapter 2** we evaluated the association of PYY (appetite suppressant) with obesity and obesity-related phenotypes in the general population. Against most previously reported data, we found that circulating PYY is not inversely associated with obesity status defined by BMI or %BF adjusting for age, sex, smoking, medication use, and menopause. Our data, the largest and most comprehensive study controlling for confounding factors, do not support the idea that adiposity status, indexed by %BF or

BMI, determines basal PYY concentrations. In addition, contrary to the initial or current literature we have demonstrated for the first time that fasting circulating PYY was positively associated with %BF, percent trunk fat (%TF), and waist circumference in women. Our study also provides strong evidence that there is an obvious sex difference regarding fasting PYY concentration and that age, smoking, medication use and menopausal status all significantly influence circulating PYY concentration in women, but not men. Although the effect size of the positive association of PYY with adiposity in women is small, and potentially negligible, it may in fact represent a protective response to weight gain.

Aside for investigating the association of appetite regulating hormones with obesity in the general population, we also sought to explore the response in circulating PYY (**Chapter 3**) and adiponectin (**Chapter 4**) in normal-weight, overweight and obese individuals to a 7-day hypercaloric diet. Regarding PYY before overfeeding we found no significant differences in fasting serum PYY concentrations between normal-weight, overweight or obese men classified by %BF (DXA) or BMI. This finding was also not surprising since the much larger study described in chapter 2 did not find any significant association of circulating PYY with obesity status in men. We also discovered that serum PYY was significantly increased due to short-term positive energy challenge independent of adiposity. The significant increase in PYY, being an appetite suppressant, could be physiologically meaningful by acting as a protective mechanism to counteract the hypercaloric diet. Regarding adiponectin before overfeeding, we also found no

significant differences in fasting adiponectin between normal-weight, overweight or obese men classified by %BF (DXA) or BMI. We did observed an inverse relationship with percent trunk fat (%TF), but this relationship is likely dependent upon insulin resistance. This is supported by the fact that after controlling for fasting insulin and/or homeostatic model assessment (HOMA-IR) the association of %TF with adiponectin was nullified. Lastly, serum adiponectin was also significantly increased due to short-term overfeeding independent of adiposity. Our data suggests that the increase in adiponectin, in the face of short-term positive energy challenge, may act as a protective mechanism during periods of weight gain against insulin resistance independent of obesity status. This was the first study to indicate that the human body preserves the ability to increase the level of adiponectin when challenged by positive energy balance in people with all range of obesity status. This finding is important for the treatment of insulin resistance in overweight/obese people.

In **Chapter 5** we revealed that the favorable effects of dietary intake of magnesium was significantly positively associated with adiposity. We discovered a beneficial dose dependent relationship between magnesium intake and insulin resistance, independent of age, gender, total caloric intake, physical activity, medication use, menopause, and adiposity. Moreover, this favorable association was more significant in overweight and obese subjects, suggesting that they may be more sensitive to the beneficial influences of dietary magnesium intake. Adding to these findings, pre-menopausal women were more significantly associated with the beneficial effects of

magnesium intake on insulin resistance than post-menopausal women. We considered, since over 68% of our post-menopausal women are medication users, that the lack of association of magnesium intake with insulin resistance could be drug related interference. However, this relationship remained absent among post-menopausal women whether or not medication status was included in the regression model. Lastly, we also provide the first evidence that the association between dietary magnesium and insulin resistance was more strongly associated with DXA %BF than BMI and the concomitant increase in DXA %BF over BMI. Therefore, because %BF more accurately represents adiposity than BMI, caution should be taken when attempting to utilize BMI as a measure of adiposity. Chapter 5 may seem slightly disconnected from the entirety of this thesis, but this could not be further from the truth. This work was vital to the development of my comprehensive understanding of the complexity of obesity research. It also demonstrates the significant influence of confounding factors when investigating obesity and obesity related co-morbidities. But more importantly it led to question the definition of obesity itself.

Our evaluation of the accuracy of the BAI method we first explored in **Chapter 6** to determine whether BAI, a new predictive equation for adiposity, was more concordant with percent body fat (%BF) measured by DXA than the BMI. We found that the entire cohort, where male and female subjects were mixed, the BAI was significantly more concordant with %BF than BMI. However, when these relationships were evaluated among men and women separately, BMI was now slightly more concordant with %BF

than BAI. Adding to these results, we were also interested in investigating the potential influence of obesity status on the predictive power of BMI and BAI. We found that the BAI was more concordant with %BF than BMI in normal-weight, overweight, and obese subjects for the entire cohort. Conversely, when evaluated among men and women separately, the BAI remained more concordant with %BF than BMI for normal-weight and overweight subjects. Therefore, BAI was found to be a better modality to predict adiposity than BMI in non-obese Caucasian populations. Our findings indicate that BAI does have some advantages over the utilization of BMI, but caution should be taken when attempting to predict adiposity with BAI equation among obese women and men. We believe the weakness of the BAI equation is that adiposity relies not only on height and hip circumference measurements alone. The BAI equation lacks the marker(s) that can reflect the change of body fat, which is critical due to the fact constant changes are the main characteristics in the development of obesity. This study strongly suggests that a measurement sensitive to the changes in adiposity for both men and women should be incorporated into a new method to increase accuracy which is exactly what we have developed and illustrated in Chapter 8.

In **Chapter 7**, we found that BMI was more significantly associated with CRFs than either BAI or %BF in the Newfoundland population independent of sex. However, the association of CRFs with BAI was more strongly concordant with %BF than BMI in both the general population and when men and women were examined separately. Regarding the severity of adiposity, the influence of increasing BAI on CRFs was more

strongly concordant with the increase in %BF than BMI. Although our results demonstrated that BAI more closely represents the association of %BF with CRFs than BMI, it is clear that BAI, like BMI, does not accommodate for the sexual dimorphism. By not taking the sex-specific differences in adiposity into consideration both the BMI and BAI equations are limited in their ability to predict adiposity and to subsequent represent the association of adiposity with CRFs. Therefore, we again have suggested that implementing measurements sensitive to the accumulation of body fat in men and women would aid in producing sex-specific equations with greater predictive power of adiposity and its true association with CRFs.

Lastly, in **Chapter 8**, since we have demonstrated that the sexual dimorphism of adiposity significantly weakens the ability of both BAI and BMI to predict adiposity we developed sex-specific equations to more accurately predict body fat percentage and better define obesity status. Our findings demonstrate that our body fat index (BFI) equations can predict %BF significantly better than BAI and BMI in the general population, and among men and women separately, at various extremes of adiposity. Additionally, having introduced waist circumference which is sensitive to weight change, we believe that our equations will be more sensitive to the change in %BF observed during weight gain/loss. The BFI_m and BFI_f equations describe in **Chapter 8** will be a valuable tool for physicians, and epidemiological researchers, wishing to accurately assess relative body fat and its association with chronic diseases.

In conclusion, this thesis has produced various novel findings and provided many new insights into understanding the etiology of obesity and diabetes development. The significant strength of the current thesis is that both cross-sectional (CODING study) and intervention (Overfeeding study) studies were performed to investigate and answered many important physical and biochemical obesity-related questions. In addition, the availability of DXA data in a population-based study gave us the ability to validate the predictive power of various adiposity equations and the subsequent development of equations that better predict adiposity. Ultimately, the goal of the thesis was to comprehensively increase our understanding of obesity and subsequently produce novel findings to further our understanding of this condition. We believe that the work enclosed has accomplished this goal.

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APPENDIX: 1 - MANUSCRIPTS PUBLISHED DURING PHD

1.1

Cahill, F., Ji, Y., Wadden, D., Amini, P., Randell, E., Vasdev, S., Gulliver, W., Sun,G. (2014) The Association of Serum Total Peptide YY (PYY) with Obesity and Body Fat Measures.**PLOS ONE - PONE-D-13-54679**

1.2

Payne, A., Abarin, T., **Cahill, F**., Loredo-Osti, J.C., Sun, G. (2014)The Effect of FTO Gene and Physical Activity Interaction on Trunk Fat Percentage Among the Newfoundland Population. **Libertas-Academica-ID-14957**

1.3

Miller, M.B., Pearcey, G.E.P., **Cahill, F**., McCarthy, H., Stratton, S.B.D., Noftall, J.C., Buckle, S., Basset, F.A,. Sun, G., Button, D.C.(2014) The effect of a short-term high intensity circuit training program on work capacity, body composition and blood profiles in sedentary obese men: a pilot study.**BioMed Research International 191797**

1.4

Cahill, F., Amini, P., Wadden, D., Khalili, S., Randell, E., Vasdev, S., Gulliver, W., Sun, G. (2013) Short-term Overfeeding Increases Circulating Adiponectin Independent of Obesity Status. **PLoS ONE 8(8): e74215. doi:10.1371/journal.pone.0074215**

1.5

Pedram, P., Wadden, D., Amini, P., Gulliver, W., **Cahill, F.,**Randell, E.,Vasdev, S., Goodridge, A., Sun, G. (2013) Food Addiction: Its Prevalence and Significant Association with Obesity in the General Population. **PLoS ONE 8(9): e74832. doi:10.1371/journal.pone.0074832**

1.6

Amini, P., **Cahill, F.,**Wadden, D., Ji, Y., Pedram, P., Sangeetha, V., Yi, Y., Gulliver, W., Paterno, G., Zhang, H., Sun, G. (2013) Beneficial association of serum ghrelin and PYY with bone mineral density in the Newfoundland population. **BMC Endocrine Disorders 2013, 13:35 doi:10.1186/1472-6823-13-35.**

1.7

Wadden, D., **Cahill F**., Amini P., Randell E., Vasdev S., Sun G. (2013)Circulating Glucagon-like peptide-1 (GLP1) Increases in Response to Short-Term Overfeeding in Men. **Nutrition & Metabolism 2013, 10:33 doi:10.1186/1743-7075-10-33**

1.8

Cahill, F., Shahidi, M., Shea. J.,Wadden, D., Gulliver, W., Randell, E., Vasdev, S., Sun, G. (2013) High Dietary Magnesium Intake is Associated with Low Insulin Resistance in the Newfoundland Population. **PLoS ONE 8(3): e58278. doi:10.1371/journal.pone.0058278**

1.9

Wadden D., **Cahill F**., Amini P., Randell E., Vasdev S., Yi Y., Zhang W., Sun G. (2012) Serum Acylated Ghrelin Concentrations in Response to Short-Term Overfeeding in Normal Weight, Overweight, and Obese Men. **PLoS ONE 7(9): e45748. doi:10.1371/journal.pone.0045748**

1.10

Amini P., Wadden D., **Cahill F**., Randell E., Vasdev S., Chen X., Gulliver W., Zhang W., Hongwei Zhang H., Yi Y., Sun G. (2012) Serum Acylated Ghrelin Is Negatively Correlated with the Insulin Resistance In the CODING study. **PLoS ONE 7(9): e45657. doi:10.1371/journal.pone.0045657**

1.11

Sun,G., **Cahill, F**., Gulliver, W., Yi,Y., Xie, Y., Bridger, T., Pace, D., Zhang, H. (2012) Concordance of BAI and BMI with DXA in the Newfoundland population.**Obesity Journal DOI: 10.1002/oby.20009**

1.12

Cahill, F., Shea, J.L., Randell, E., Vasdev, S., Sun,G. (2010) Whole PYY response is blunted in overweight & obese young men after overfeeding.**American Journal Clinical Nutrition - AJCN/2010/003624**

APPENDIX: 2- ABSTRACTS PUBLISHED DURING PHD

2.1

Cahill,F., Amini, P., Wadden, D., Ji, Y., Khalili, S., Randell, E., Vasdev, S., Sun, G. **(2013)** The Response and Interaction of Adiponectin, Leptin, and TNF-α Due to a Short-term Positive Energy Challenge. **31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1754590**

2.2

Cahill, F., Wadden, D., Amini, P., Bridger, T., Gulliver, W., Sun, G. **(2013)** Significant Association of Dietary Macronutrient Intake with Serum Gastrointestinal Hormones in Obese Children. (2013) **31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1754611**

2.3

Amini, P., D, Wadden, D., **Cahill, F.,**Pedram, P., Vidyasankar, S., Gulliver, W., Randell, E., Zhang, H., Sun, G. **(2013)**Serum Acylated Ghrelin Is Negatively Correlated with High Sensitivity C - Reactive Protein in the Newfoundland Population. **31st Annual Obesity Society Scientific Meeting. CONTROL ID:1753813**

2.4

Amini, P.,**Cahill, F.,**Wadden, D., Pedram, P., Vidyasankar, S., Gulliver, W., Randell, E., SunG. **(2013)**High Dietary Selenium Intake Is Associated with a Low Percentage of Body Fat in the Newfoundland Population.**31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1753464**

2.5

Amini, P., **Cahill, F.,**Wadden, D., Pedram, P., Gulliver, W., Randell, E., Bridger, T., Zhang, H., Sun, G. **(2013)**Correlation of Gut Hormones with Body Composition Characteristics in Obese Children.**31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1754541**

2.6

Pedram, P., Amini, P., Wadden, D., **Cahill, F.**, Gulliver, W., Zhai, G., Sun, G. **(2013)** Hormonal, Metabolic and Dietary Characteristics in Food Addicted Obese.**31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1753151**

2.7

Pedram, P., Wadden, D., Amini, P., Gulliver, W., Randell, E., **Cahill, F**., Vasdev, S., Goodridge, A., Carter, J., Zhai, G., Sun, G. **(2013)** Food Addiction: Its Prevalence and Significant Association with Obesity in the General Population.**31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1753322**

2.8

Wadden, D., Amini, P., **Cahill, F.,**Pedram, P., Bridger, T., Gulliver, W., Randell, E., Sun, G. **(2013)** Gut Hormones and Childhood Obesity in the Newfoundland Population.**31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1754596**

2.9

Wadden, D., Rideout, A., **Cahill, F.,**Amini, P., Vidyasankar, S., Randell, E., Gulliver, W., Sun, G. **(2013)** Dietary BCAA Intake Is Inversely Correlated with Insulin Resistance in the Newfoundland Population. **31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1753131**

2.10

Miller, M.B., Pearcey, G.E.P., **Cahill, F**., Basset, F.A,. Stratton, S.B.D., Sun, G., Button, D.C.**(2013)**Six hours total of high intensity circuit training improves key physiological health markers in obese males.**Canadian Society for Exercise Physiology (CSEP)**

2.11

Cahill, F., Wadden, D., Amini, P., Lee, A., Randell, E., Vasdev, S., Sun, G.**(2012)** Pancreatic Polypeptide (PP) Response to a 7-Day Overfeeding in Young Men. **30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374895**

2.12

Cahill, F., Wadden, D., Amini, P., Lee, A., Yi, Y., Randell, E., Sun, G.**(2012)**The Response of Five Functionally Connected Gut Hormones (PP, PYY, CCK, GLP-1 and Ghrelin) to a Short-term Positive Energy Challenge. **30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1375761**

2.13

Cahill, F., Wadden, D., Amini, P., Lee, A., Yi, Y., Randell, E., Vasdev, S., Sun, G. **(2012)**Association Between the Body Adiposity Index (BAI) and Cardiometabolic Risk Factors (CRFs) among normal-weight (NW), overweight (OW), and obese (OB) men and women in the CODING study. **30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374824**

2.14

Wadden, D., Amini, P., **Cahill, F**., Lee, A., Vasdev, S., Randell, E., Gulliver, W., Sun, G. **(2012)**Circulating Ghrelin is Inversely Associated with Dietary Sugar Intake in the Newfoundland **Population. 30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374806**

2.15

Wadden, D., **Cahill, F**., Randell, E., Yi, Y., Vasdev, S., Sun, G. **(2012)**GLP-1 Response to Short-term Overfeeding in Young Men. **30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374730**

2.16

Wadden, D., **Cahill, F**., Vasdev, S., Randell, E., Gulliver, W., Sun, G. **(2012)**Dietary Leucine is Associated With Reduced Insulin Resistance in Women in the Newfoundland Population. **30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374770**

2.17
Amini, P., Wadden, D., Vidyasankar, **Cahill, F**., S., Gulliver, W., Zhang, H., Sun, G. **(2012)**Circulating ghrelin is associated with alcohol consumption in the CODING study. **30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374745**

2.18
Amini, P., **Cahill, F**., Wadden, D., Randell, E., Vasdev, S., Sun, G. **(2012)**Short term positive energy challenge does not change fasting serum cholecystokinin level. **30th Annual Obesity Society Scientific Meeting.CONTROL ID: 1374890**

2.19
Cahill,**F**., Wadden,D., Gulliver,W., Randell,E., Vasdev,S., Sun, G. **(2011)**NoAssociation Between Serum Whole Peptide YY (PYY) and Adiposity Status was found in the NewfoundlandPopulation. **29th Annual Obesity SocietyScientific Meeting ABSTRACT ID: 507-P**

2.20
Cahill,**F**., Wadden,D., Gulliver,W., Randell,E., Vasdev,S., Sun, G.**(2011)**FastingSerum Peptide YY (PYY) Concentration is Associated with Glucose and Insulin Resistance in pre-menopausalWomen. **29th Annual Obesity Society Scientific MeetingABSTRACTID: 517-P**

2.21
Cahill,**F**., Wadden,D., Gulliver,W., Randell,E., Vasdev,S., Sun, G.**(2011)**Serumwhole Peptide YY (PYY) is Inversely Associated with Dietary Protein and Essential Amino Acids (EAAs) for Menin the CODING STUDY. **29th Annual Obesity Society Scientific MeetingABSTRACTID: 518-P**

2.22
Wadden,D., **Cahill**,**F**., Gulliver,W., Sun, G.**(2011)** Higher Dietary Essential Amino Acid Intake isAssociated with Lower Body Fat. **29th Annual Obesity Society Scientific MeetingABSTRACTID: 539-P**

2.23
Wadden,D., **Cahill**,**F**., Xie, Y., Gulliver,W., Zhang, H., Sun, G.**(2011)**Significant reverse correlation between dietary intakes of three branched essential amino acids (Leu, IIe and Val)and percent body fat in the Newfoundland population. **12th ICAA, Pep and protein.**

2.24
Sun, G., **Cahill, F.,** Yi.Y.,Xie, Y., Bridger, T., Pace, D., Zhang, H. **(2011)**Concordance of Body Adiposity Index (BAI) and BMI with DXA.**29th Annual Obesity Society Scientific MeetingABSTRACTID: 774-P**

2.25
Sun, G., Sheridan, K.,**Cahill, F.,**Xie, Y., Randell,E., Vasdev,S., Pace, D., Gulliver,W.,Zhang, H.**(2011)** Fasting serum acylated ghrelin level is not associated with body composition inthe Newfoundland population. **29th Annual Obesity Society Scientific MeetingABSTRACTID: 512-P**

2.26
Sun, G., Sheridan, K.,**Cahill, F.,**Vasdev,S., Randell,E., Gulliver,W.,Zhang, H. **(2011)** Serum acylated ghrelin is negatively associated dietary protein and essential amino acids in the CODINGstudy. **29th Annual Obesity Society Scientific MeetingABSTRACTID: 515-P**

2.27
Sun, G., Sheridan, K., **Cahill, F.,**Vasdev,S.,Randell,E., Gulliver,W.,Zhang, H.**(2011)** Serum acylated ghrelin is inversely correlated with insulin resistance in the CODING study. **29th Annual Obesity Society Scientific MeetingABSTRACTID: 508-P**

2.28

Cahill, F., Sheridan K., Randell, E., Vasdev, S., Sun,G. **(2010)**Peptide YY (PYY) Responses to a 7-Day Positive Energy Challenge in Normal-Weight,Overweight, and Obese Young Men. **28th Annual Obesity Society Scientific MeetingABSTRACTID: 762-P**

2.29

Sheridan K., **Cahill, F.,** Randell, E., Vasdev, S., Sun,G. **(2010)** Ghrelin concentrations in response to short-term overfeeding in Normal-Weight, Overweight, and Obese Men. **28th Annual Obesity Society Scientific MeetingABSTRACTID: 757-P**

APPENDIX: 3 -POSTERS PRESENTED AT INTERNATIONAL CONFERENCES

A3.1 - Peptide YY (PYY) Responses to a 7-Day Positive Energy Challenge in Normal-Weight, Overweight, and Obese Young Men.

Cahill, F., Sheridan K., Randell, E., Vasdev, S., Sun,G. (2010) 28th Annual Obesity Society Scientific Meeting ABSTRACT ID: 762-P

Background: Gut hormones are important factors affecting food intake and the maintenance of energy homeostasis through gut-brain communication. Peptide YY (PYY), a linear 36amino acid, inhibits appetite and has been linked to the development of obesity. **Objective:** Our study was to investigate the relationship between PYY and obesity related phenotypes before and after a 7-day overfeeding protocol (70% above normal energy requirement). **Design:** The study recruited 72 participants. We analyzed the potential relationship between serum PYY before and after overfeeding and the PYY changes with baseline energy requirement measured by the Willet Frequent Food Questionnaire and three 24hr recalls, weight, BMI, %BF, waist circumference, glucose, insulin, insulin resistance and beta cell function evaluated by the Homeostasis Model Analysis (HOMA-IR, HOMA-β), total cholesterol, HDL, LDL. **Results:** The PYY concentration was significantly increased after the 7-day positive energy challenge (118.3pg/ml ± 57.8 to 135.8pg/ml ± 70.1, $p = 0.010$). However, no significant differences of serum PYY between normal weight, overweight and obese subjects based on BMI or Body Fat Percentage (%BF) measured by DXA. No significant correlation was found after controlling for age and %BF or BMI. The positive energy challenge was overwhelmingly strong and induced significant change in body composition related phenotypes, negative changes in lipids and insulin resistance in the entire cohort. **Conclusion:** To our knowledge, this is the first study on the regulation of PYY by positive energy challenge. The increase in PYY, likely a protective response to overfeeding, suggests its usefulness in the prevention/treatment of human obesity.

Table 1. Physical and biochemical characteristics of subjects at baseline and in response to 7-days of overfeeding[1]

	Normal-Weight (n = 23 - 27)		Overweight (n = 14)		Obese (n = 23 - 28)	
	Before	**After**	**Before**	**After**	**Before**	**After**
Age (y)	23.72 ± 3.6	NA	21.97 ± 3.1	NA	23.24 ± 2.6	NA
Height (cm)	179.51 ± 6.4	NA	179.38 ± 4.6	NA	179.07 ± 6.8	NA
Weight (kg)[4,8]	72.39 ± 9.2	74.53 ± 9.6†	77.81 ± 4.2	79.39 ± 4.3†	93.01 ± 15.6	95.65 ± 16.0†
BMI (kg/m^2) [4,8]	22.55 ± 2.6	23.23 ± 2.8†	24.13 ± 1.3	24.63 ± 1.5†	29.10 ± 4.9	29.93 ± 5.0†
Percent Body Fat (%) [3,6]	14.63 ± 3.3	15.38 ± 3.4†	22.54 ± 0.8	22.82 ± 1.1†	31.51 ± 5.0	31.26 ± 4.7
Percent Trunk Fat (%) [3,6]	16.55 ± 3.6	17.52 ± 3.8†	25.39 ± 1.8	25.79 ± 2.2†	35.22 ± 5.4	34.89 ± 5.1
Percent Android Fat (%) [3,6]	19.01 ± 4.4	19.86 ± 4.9†	28.84 ± 2.6	29.45 ± 2.7	40.47 ± 7.1	41.06 ± 6.7
Total Cholesterol (mmol/L)	4.39 ± 0.9	4.67 ± 0.8†	4.63 ± 0.9	4.72 ± 1.1	4.56 ± 0.7	4.79 ± 0.8†
HDL - Cholesterol (mmol/L) [4,7]	1.38 ± 0.3	1.47 ± 0.3†	1.38 ± 0.3	1.43 ± 0.3	1.19 ± 0.2	1.31 ± 0.3†
LDL - Cholesterol (mmol/L)	2.61 ± 0.7	2.67 ± 0.7	2.82 ± 0.7	2.83 ± 0.9	2.79 ± 0.7	2.79 ± 0.6
Triacylglycerol (mmol/L)[5,8]	0.90 ± 0.3	1.16 ± 0.8†	0.92 ± 0.3	1.00 ± 0.5	1.37 ± 0.7	1.58 ± 0.9
Glucose (mmol/L)	4.97 ± 0.4	5.03 ± 0.5	5.03 ± 0.4	5.09 ± 0.6	5.28 ± 0.7	5.17 ± 0.5
Insulin (pmol/L) [4,7]	42.86 ± 23.5	62.98 ± 23.8†	69.51 ± 69.16	88.85 ± 86.3	96.99 ± 91.8	111.97 ± 77.1†
HOMA-IR[4,7]	1.39 ± 0.8	2.09 ± 0.9†	2.35 ± 2.68	2.95 ± 2.9	3.51 ± 3.8	3.85 ± 2.9
HOMA-β[4,7]	83.32 ± 38.6	125.63 ± 49.4†	120.21 ± 74.09	175.90 ± 163.6	147.49 ± 90.62	189.67 ± 103.4†
Peptide YY (pg/mL)	121.47 ± 60.8	148.44 ± 93.37†	109.90 ± 53.8	119.49 ± 38.9	118.93 ± 56.54	136.47 ± 58.27

[1] PYY, PYY, Peptide YY; HOMA-IR, Homeostasis model assessment of insulin resistance; HOMA-β of β cell function; NA, not applicable.

[2] Adiposity status and response to overfeeding were analyzed by using a 2-factor mix model ANOVA for repeated measures (SPSS, version 17.0 Chicago, IL, USA).

[3] Significant differences between normal-weight, overweight and obese subjects at baseline (2-Way mixed model ANOVA).

[4] Significant difference between normal-weight vs obese subjects at baseline (2-Way mixed model ANOVA).

[5] Significant difference between obese vs normal-weight and obese vs overweight subjects at baseline (2-Way mixed model ANOVA).

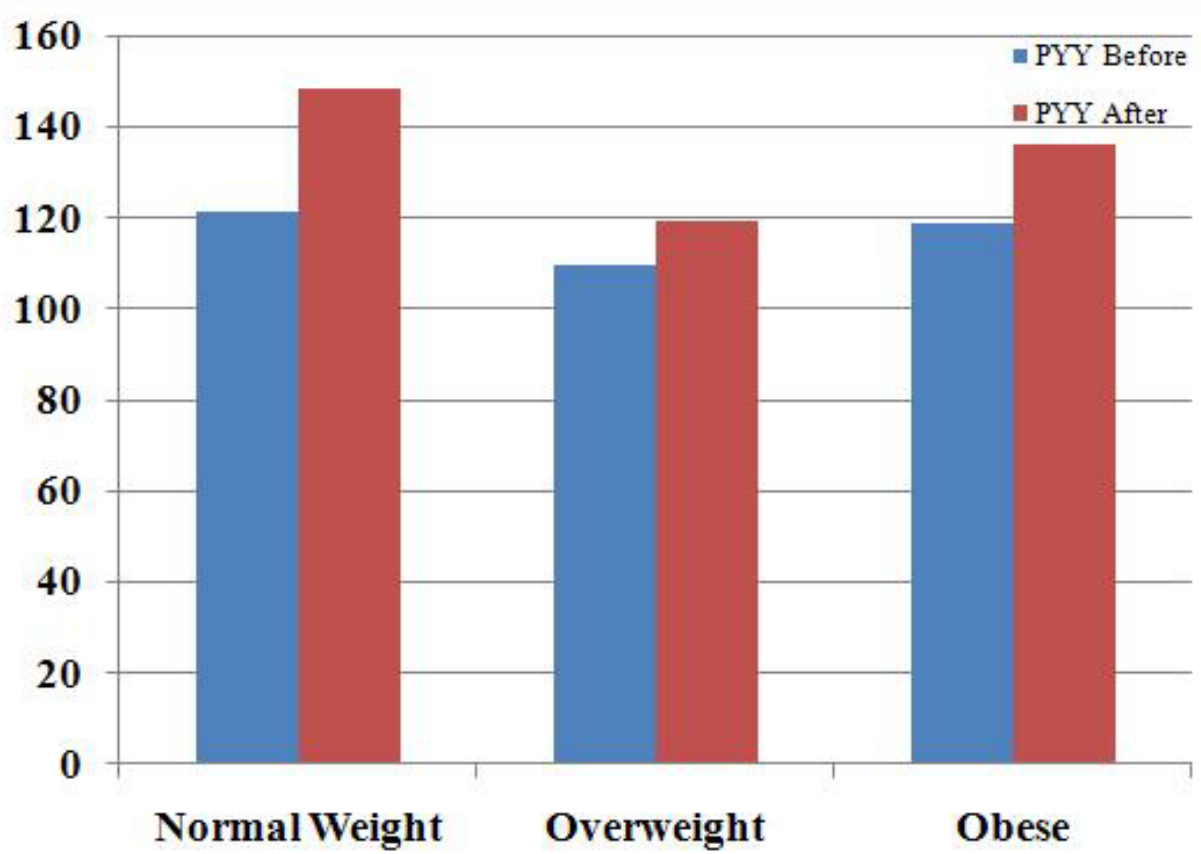
[6] Significant differences between normal-weight, overweight and obese subjects due to overfeeding (2-Way mixed model ANOVA).

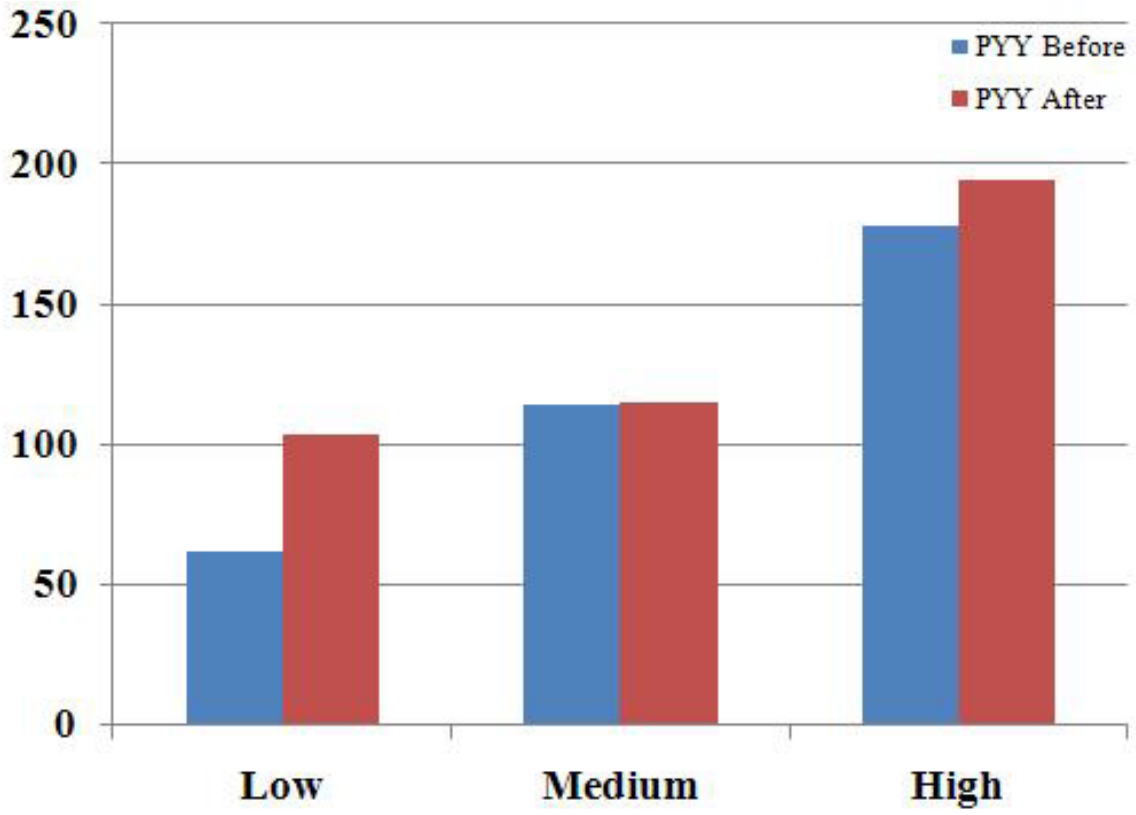
[7] Significant difference between normal-weight vs obese subjects due to overfeeding (2-Way mixed model ANOVA).

[8] Significant difference between obese vs normal-weight and obese vs overweight subjects due to overfeeding (2-Way mixed model ANOVA).

[9] Significant level was set to $p < 0.05$.

† Significant difference within group (Paired *t*-test).


160
140
120
100
80
60
40
20
0
PYY Before
PYY After
Normal Weight
Overweight
Obese


250
200
150
100
50
0
PYY Before
PYY After
Low
Medium
High

A3.2 - Concordance of Body Adiposity Index (BAI) and BMI with DXA.

Sun, G., Cahill, F., Yi.Y.,Xie, Y., Bridger, T., Pace, D., Zhang, H. (2011) 29th Annual Obesity Society Scientific Meeting ABSTRACT ID: 774-P

Background: A recent study created a new parameter called Body Adiposity Index (BAI) to evaluate body fat using hip circumference and height. The equation was developed using a Mexican-American population and has not been validated in any Caucasian population.

Objective: Compare the BAI and BMI by comparing the concordance with the dual-energy x-ray absorptiometry (DXA). **Design:** A total of 2601 adults from the CODING study recruited from the Canadian Province of Newfoundland & Labrador were used in the analysis. The partial correlation analysis was used. Comparison was performed in all subjects and in men and women separately. In addition, body fat percentage (%BF) was also compared. **Results:** The correlation coefficients of BAI and BMI with DXA were r = 0.78 and r = 0.56 respectively for the entire cohort. However, when analysis was performed according to gender the performance of BAI compared with BMI was lower (r = 0.67 BAI vs. r = 0.70 BMI and r = 0.74 BAI vs. r = 0.76 in men and women respectively). **Conclusion:** The performances of BAI for %BF were lower in obese men and women when analysis was performed according to sex. BAI is a simple and better method than BMI in evaluating adiposity in NW and OW people but worse than BMI in OB people. We would suggest that a measurement sensitive to the changes in adiposity for both men and women be incorporated into the present BAI equation to increase efficiency

Table .1 Composition characteristics of subjects.

	Entire Cohort (n = 2601)		Male (n = 662)		Female (n = 1939)		
	Mean ± SD		Mean ± SD		Mean ± SD		P
Age [3]	42.34	± 13.05	39.55	± 14.4	43.30	± 12.4	0.000
Weight (kg) [2]	73.28	± 15.53	84.70	± 14.3	69.38	± 13.9	0.000
Height (cm) [2]	165.98	± 8.65	176.37	± 6.6	162.43	± 6.0	0.000
Waist (cm) [2]	91.35	± 14.27	96.09	± 13.3	89.73	± 14.2	0.000
Hip (cm) [3]	100.34	± 11.65	99.14	± 9.9	100.74	± 12.2	0.002
BMI (kg/m^2) [2]	26.55	± 4.99	27.23	± 4.3	26.31	± 5.2	0.000
BAI [3]	29.16	± 6.62	24.41	± 4.6	30.78	± 6.4	0.000
Body Fat (%) [3]	34.06	± 9.56	24.52	± 7.9	37.32	± 7.7	0.000

[1] All values are means ± SDs. Gender differences were analyzed by an independent t-test.

[2] Variable significantly greater in men

[3] Variable significantly greater in women

[4] Significance level for t-tests were set to $p \leq 0.05$

Table. 2 Pearson correlation between both BAI and BMI with body composition measurements within the entire cohort[1]

	Entire Cohort				Male				Female			
	BMI		BAI		BMI		BAI		BMI		BAI	
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.19	0.00	0.37	0.00	0.25	0.00	0.42	0.00	0.19	0.00	0.34	0.00
Weight (kg)	0.86	0.00	0.35	0.00	0.89	0.00	0.55	0.00	0.93	0.00	0.68	0.00
Height (cm)	-0.04	NS	-0.55	0.00	-0.11	0.00	-0.41	0.00	-0.14	0.00	-0.39	0.00
Waist (cm)	0.83	0.00	0.62	0.00	0.81	0.00	0.75	0.00	0.84	0.00	0.80	0.00
Hip (cm)	0.83	0.00	0.84	0.00	0.79	0.00	0.86	0.00	0.85	0.00	0.90	0.00
Body Fat (%)	0.56	0.00	0.78	0.00	0.70	0.00	0.67	0.00	0.76	0.00	0.74	0.00

[1] Pearson correlation analysis was used to determine the relationships between both BAI and BMI with body composition measurements.

[2] Significance levels were set to $p \leq 0.05$

Table. 3 Pearson correlation between both BAI and BMI with body composition measurements among NW, OW, OB men and women[1]

	Entire Cohort				Male				Female			
	Normal Weight (n= 849)				Normal Weight (n = 244)				Normal Weight (n = 605)			
	BMI		BAI		BMI		BAI		BMI		BAI	
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.19	0.00	0.50	0.00	0.34	0.00	0.49	0.00	0.22	0.00	0.49	0.00
Weight (kg)	0.78	0.00	-0.17	0.00	0.81	0.00	0.25	0.00	0.82	0.00	0.26	0.00
Height (cm)	0.15	0.00	-0.63	0.00	-0.13	0.05	-0.45	0.00	-0.15	0.00	-0.49	0.00
Waist (cm)	0.70	0.00	0.28	0.00	0.72	0.00	0.61	0.00	0.63	0.00	0.54	0.00
Hip (cm)	0.57	0.00	0.65	0.00	0.55	0.00	0.76	0.00	0.59	0.00	0.81	0.00
Body Fat (%)	0.05	NS	0.65	0.00	0.46	0.00	0.51	0.00	0.51	0.00	0.51	0.00
	Overweight (n = 776)				Overweight (n = 177)				Overweight (n = 599)			
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.25	0.00	0.41	0.00	0.18	0.01	0.50	0.00	0.29	0.00	0.47	0.00
Weight (kg)	0.78	0.00	-0.15	0.00	0.81	0.00	0.16	0.04	0.82	0.00	0.29	0.00
Height (cm)	0.08	0.02	-0.67	0.00	-0.10	NS	-0.46	0.00	-0.19	0.00	-0.55	0.00
Waist (cm)	0.70	0.00	0.32	0.00	0.56	0.00	0.60	0.00	0.69	0.00	0.62	0.00
Hip (cm)	0.59	0.00	0.69	0.00	0.47	0.00	0.77	0.00	0.65	0.00	0.80	0.00
Body Fat (%)	-0.09	0.01	0.62	0.00	0.18	0.02	0.42	0.00	0.43	0.00	0.49	0.00
	Obese (n = 976)				Obese (n = 241)				Obese (n = 735)			
	r	P	r	P	r	P	r	P	r	P	r	P
Age	0.10	0.00	0.29	0.00	0.15	0.02	0.34	0.00	0.08	0.03	0.22	0.00
Weight (kg)	0.80	0.00	0.20	0.00	0.86	0.00	0.43	0.00	0.89	0.00	0.54	0.00
Height (cm)	-0.08	0.01	-0.62	0.00	-0.10	NS	-0.49	0.00	-0.10	0.01	-0.41	0.00
Waist (cm)	0.73	0.00	0.49	0.00	0.71	0.00	0.61	0.00	0.76	0.00	0.72	0.00
Hip (cm)	0.79	0.00	0.80	0.00	0.79	0.00	0.81	0.00	0.79	0.00	0.85	0.00
Body Fat (%)	0.34	0.00	0.68	0.00	0.51	0.00	0.40	0.00	0.58	0.00	0.54	0.00

[1] Pearson correlation analysis was used to determine the relationships between both BAI and BMI with body composition measurements. NW, Normal-weight; OW, Overweight; OB, Obese. Subjects were classified on the basis of percentage body fat as Normal-weight, overweight, or obese according to criteria recommended by Bray (18)

[2] Significance levels were set to $p \leq 0.05$

A3.3 - No Association Between Serum Whole Peptide YY (PYY) and Adiposity Status was Found in the Newfoundland Population.

Cahill,F.,Wadden,D., Gulliver,W., Randell,E., Vasdev,S., Sun, G. (2011) 29th Annual Obesity Society Scientific Meeting ABSTRACT ID: 507-P

Background: PYY is an appetite suppressing gut hormone, and has been linked to obesity in humans. However, there is little data regarding the association between PYY with obesity at the population level. **Objective:** The present study explored the link between serum PYY and obesity related phenotypes among normal-weight(NW),overweight(OW) and obese(OB) men and women. **Design:** 2358 CODING study subjects (Male=594 Female=1764) participated in this study. Whole PYY was measured with an enzyme-linked immunosorbent assay (ELISA). Adiposity status (NW, OW & OB) was determined by the Bray Criteria (age and gender specific) according to percent body fat (%BF) measured by Dual-Energy X-ray Absorptiometry (DXA). Percent trunk fat (%TF), android fat (%AF) and gynoid fat (%GF) were also measured by DXA . Partial correlations and ANOVAs were used to determine relationships with and differences of PYY within and between adiposity groups. Age and physical activity were controlled for. Analyses were also done according to menopausal, smoking, and medication use status. All subjects fasted 12 hr before blood drawings and DXA scans. **Results:** Serum PYY was higher in males than females (118.5±77.3 pg/ml vs 108.4±73.7pg/ml, p=0.001). No significant difference in serum PYY among NW, OW and OB subjects in the entire cohort. No significant difference in serum PYY among NW, OW and OB within each gender. No significant correlations between PYY and any obesity measure including BMI. **Conclusion:** To our knowledge, this is the first and largest population based study exploring the association of serum PYY with obesity related phenotypes. Our data do not support the idea that PYY plays a major role in determining adiposity status.

Table 1 - Body composition characteristics for men and women.

	Entire Cohort (n = 2358 - 2332)		Male (n = 595 - 583)		Female (n = 1764 - 1749)		
	Mean	SD	Mean	SD	Mean	SD	P
Age (y) [3]	42.34 ±	13.0	39.23 ±	14.4	43.39 ±	12.3	P < 0.001
Weight (kg) [2]	73.23 ±	15.9	85.16 ±	15.3	69.22 ±	14.0	P < 0.001
Height(cm) [2]	165.77 ±	8.6	176.12 ±	6.6	162.29 ±	5.9	P < 0.001
BMI (kg/m^2) [2]	26.34 ±	5.0	27.35 ±	4.6	26.01 ±	5.1	P < 0.001
Waist (cm) [2]	91.25 ±	14.5	96.53 ±	14.0	89.48 ±	14.2	P < 0.001
Hip (cm) [3]	100.43 ±	12.0	99.39 ±	10.5	100.77 ±	12.4	P < 0.02
Body fat (%) [3]	34.28 ±	9.4	24.94 ±	8.0	37.41 ±	7.6	P < 0.001
Trunk fat (%) [3]	36.40 ±	9.8	29.70 ±	9.5	38.66 ±	8.8	P < 0.001
Andriod fat (%) [3]	41.62 ±	11.4	35.84 ±	11.7	43.57 ±	10.6	P < 0.001
Gynoid fat (%) [3]	40.58 ±	9.9	28.56 ±	8.1	44.61 ±	6.6	P < 0.001
Peptide YY (pg/ml) [2]	110.99 ±	74.7	118.54 ±	77.3	108.44 ±	73.7	P < 0.005

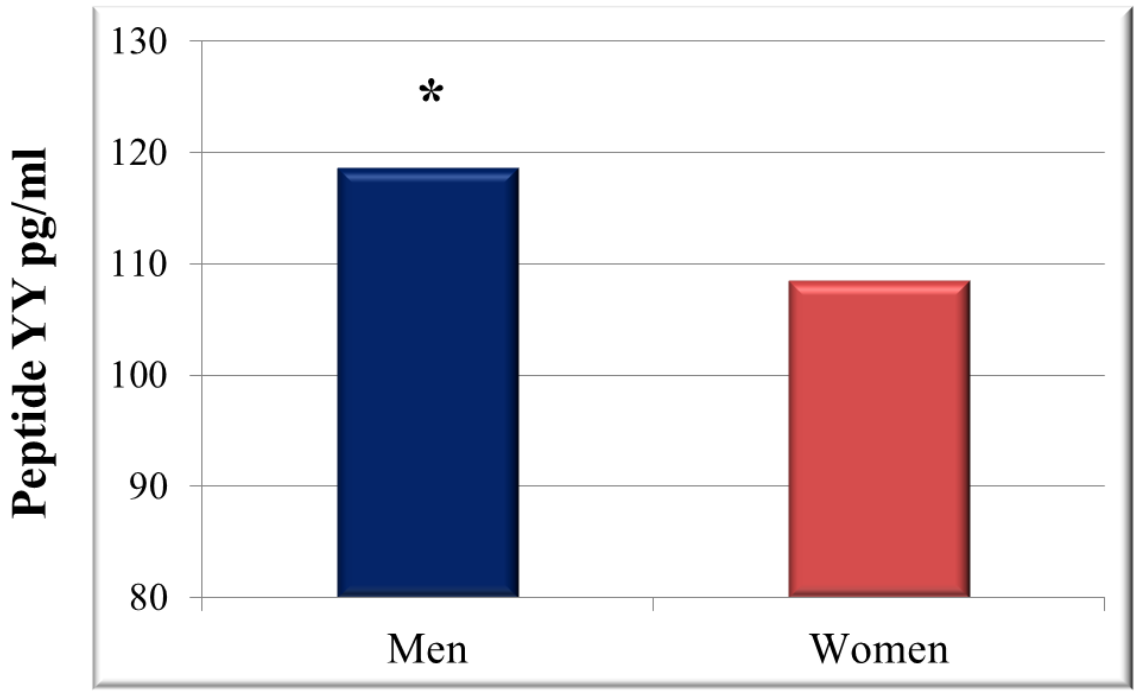
[1] All values are means ± SDs. Gender differences were analyzed by an independent t-test.

[2] Variable significantly greater in men than women.

[3] Variable significantly greater in women than men.

[4] Significant level for t-tests were set to $P \leq 0.05$.

Figure 1 - PYY Concentration Between Genders



* Significance level for t-test was set to $P \leq 0.05$

Table 2 – Body composition characteristics of normal-weight, overweight and obese subjects.

	Male				Female			
	Normal	Overweight	Obese		Normal	Overweight	Obese	
	(n = 197 - 200)	(n = 152 - 156)	(n = 234 - 236)		(n = 506 - 508)	(n = 541 - 543)	(n = 702 - 711)	
Age (y)	34.71 ± 14.84	44.13 ± 12.80	39.85 ± 13.90	1v2v3	40.04 ± 13.39	44.90 ± 12.00	44.64 ± 11.24	1v2&3
Weight (kg)	74.53 ± 9.57	84.45 ± 10.32	94.64 ± 15.91	1v2v3	58.27 ± 6.22	65.76 ± 7.93	79.69 ± 14.34	1v2v3
Height(cm)	176.77 ± 6.85	175.38 ± 5.47	176.07 ± 7.09	NS	163.06 ± 5.64	162.06 ± 5.96	161.91 ± 6.06	1v2&3
BMI (kg/m^2)	23.88 ± 2.73	27.41 ± 3.11	30.25 ± 4.57	1v2v3	21.67 ± 2.07	24.73 ± 2.89	30.08 ± 4.99	1v2v3
Waist (cm)	85.53 ± 8.02	95.97 ± 8.23	106.20 ± 13.95	1v2v3	78.04 ± 7.48	87.06 ± 9.56	99.61 ± 13.91	1v2v3
Hip (cm)	91.82 ± 7.28	98.86 ± 7.37	106.12 ± 10.18	1v2v3	90.43 ± 6.81	98.49 ± 7.36	109.99 ± 11.88	1v2v3
Waist-Hip Ratio	0.93 ± 0.06	0.97 ± 0.04	1.00 ± 0.08	1v2v3	0.86 ± 0.07	0.88 ± 0.07	0.90 ± 0.07	1v2v3
Body fat (%)	16.06 ± 4.56	25.06 ± 2.76	32.37 ± 4.09	1v2v3	28.13 ± 4.63	36.83 ± 2.29	44.48 ± 3.70	1v2v3
Trunk fat (%)	19.13 ± 6.24	30.76 ± 4.09	37.95 ± 4.44	1v2v3	28.07 ± 5.68	38.40 ± 3.72	46.43 ± 4.41	1v2v3
Andriod fat (%)	23.80 ± 9.60	37.45 ± 5.94	45.07 ± 5.53	1v2v3	31.67 ± 8.30	43.31 ± 5.51	52.34 ± 5.26	1v2v3
Gynoid fat (%)	21.56 ± 6.99	27.78 ± 4.53	35.07 ± 4.89	1v2v3	38.06 ± 5.17	44.09 ± 3.74	49.74 ± 4.64	1v2v3
Peptide YY (pg/ml)	119.68 ± 81.20	122.24 ± 71.52	114.92 ± 77.96	NS	104.07 ± 70.52	112.64 ± 75.77	108.50 ± 74.20	NS

[1] All values are means ± SDs. NW, normal-weight; OW, overweight; OB, obese. Subjects were classified on the basis of percentage body fat as normal weight, overweight, or obese according to criteria recommended by Bray . Adiposity status was analyzed by one-way ANOVA. [2] 1v2v3 (1 = NW, 2 = OW, 3 = OB) = OB greater then NW and OW with OW being greater then NW. [3] 1v2&3 (1 = NW, 2 = OW, 3 = OB) = NW less then OW and OB. [4] Significant level for one-way ANOVA was set to $P \leq 0.05$.

Table 3 – Partial correlations of serum PYY concentrations with body composition characteristics

	Male						Female					
	Normal Weight (n = 197 - 200)		Overweight (n = 152 = 156)		Obese (n = 234 = 236)		Normal Weight (n = 506 - 508)		Overweight (n = 541 - 543)		Obese (n = 702 - 711)	
	r	P	r	P	r	P	r	P	r	P	r	P
Weight (kg)	-0.05	NS	0.14	NS	-0.07	NS	0.00	NS	-0.03	NS	0.02	NS
Height(cm)	-0.04	NS	0.15	NS	-0.02	NS	0.01	NS	0.03	NS	0.02	NS
BMI (kg/m^2)	-0.04	NS	0.08	NS	-0.05	NS	-0.02	NS	-0.05	NS	0.02	NS
Waist (cm)	-0.02	NS	0.17	NS	-0.05	NS	0.00	NS	-0.03	NS	0.09	NS
Hip (cm)	-0.06	NS	0.11	NS	-0.11	NS	-0.02	NS	-0.04	NS	0.05	NS
Body fat (%)	-0.04	NS	0.07	NS	-0.05	NS	-0.08	NS	0.02	NS	0.02	NS
Trunk fat (%)	-0.02	NS	0.02	NS	-0.05	NS	-0.04	NS	0.06	NS	0.02	NS
Andriod fat (%)	0.02	NS	0.04	NS	-0.05	NS	0.01	NS	0.05	NS	-0.02	NS
Gynoid fat (%)	-0.03	NS	0.11	NS	-0.07	NS	-0.09	NS	-0.02	NS	-0.06	NS

* **Significance level for partial correlations was set to $P \leq 0.05$**

A3.4 - Serum whole Peptide YY (PYY) is Inversely Associated with Dietary Protein and Essential Amino Acids (EAAs) for Men in the CODING STUDY.

Cahill, F.,Wadden, D., Gulliver, W., Randell, E., Vasdev, S., Sun, G.(2011) 29th Annual Obesity Society Scientific Meeting ABSTRACT ID: 518-P

Background: PYY is a gut hormone which encourages the cessation of food intake. It has been suggested that serum levels of PYY is related with dietary protein. However, data is missing regarding the association between macro-nutrient intake with circulating PYY in the general population. **Objective:** Therefore, the purpose of this study was to investigate the relationship between PYY with dietary protein, carbohydrates, fat, EAAs and total caloric intake. **Design:** 2295 CODING study subjects (Male = 578, Female = 1717) participated in this study. Whole PYY was measured using an enzyme-linked immunosorbent assay (ELISA) method. Dietary intakes of the nutrients were collected from the Willett Food Frequency survey and rendered with the Nutribase 8 software package. ANCOVA analysis, controlling for age and physical activity (PA), was performed to determined differences between gender and medication status [Medication (MED) and non-Medication (NON-MED) user]. All subjects fasted 12 hr before blood drawings and DXA scans. **Results:** Men had higher levels of PYY than women (Men 118.00±77.4pg/ml, vs. Women 108.41±73.8 pg/ml, $p = 0.001$) but women ingested more dietary protein (Men 1.30±0.96 g/kg/day vs Women=1.33±0.88g/kg/day, $p = 0.01$) and EAAs than men. However, Partial correlations (controlling age and PA) revealed a negative relationship between PYY with protein(g/kg/day) intake($r = -0.11$, $p = 0.02$) for men. Regarding medication use, NON-MED men revealed stronger associations between PYY with protein(g/kg/day)($r = -0.14$, $p = 0.01$) and a new association with 9 EAAs(mg/kg/day) ($r = -0.15$ to -0.14, $p = 0.01$ to <0.001). **Conclusions:** Our study found significant inverse correlations between serum PYY with dietary protein and 9 EAAs in men but not women. The effect of dietary protein on serum PYY could be interfered by medication use.

Table 1 – Nutritional and body composition characteristics.

	Entire Cohort (n = 2295-2280)		Men (n = 578 - 573)		Women (n = 1717 - 1707)		
	Mean	SD	Mean	SD	Mean	SD	P
Age (y) [3]	42.37 ±	12.9	39.30 ±	14.3	43.40 ±	12.3	P < 0.001
Weight (kg) [2]	73.20 ±	15.9	85.12 ±	15.3	69.19 ±	14.0	P < 0.001
Height(cm) [2]	165.77 ±	8.6	176.09 ±	6.6	162.29 ±	6.0	P < 0.001
BMI (mg/m^2) [2]	26.33 ±	5.0	27.34 ±	4.6	25.99 ±	5.1	P < 0.001
Waist (cm) [2]	91.28 ±	14.5	96.50 ±	14.0	89.52 ±	14.3	P < 0.001
Hip (cm) [3]	100.47 ±	11.9	99.37 ±	10.6	100.84 ±	12.3	P < 0.02
Body fat (%) [3]	34.27 ±	9.5	24.94 ±	8.0	37.41 ±	7.7	P < 0.001
Trunk fat (%) [3]	36.40 ±	9.8	29.72 ±	9.5	38.65 ±	8.9	P < 0.001
Andriod fat (%) [3]	41.62 ±	11.4	35.87 ±	11.7	43.55 ±	10.6	P < 0.001
Gynoid fat (%) [3]	40.58 ±	9.9	28.60 ±	8.1	44.61 ±	6.6	P < 0.001
Peptide YY (pg/ml) [2]	110.82 ±	74.8	118.00 ±	77.4	108.41 ±	73.8	P < 0.005
Protein (g/kg)[3]	1.31 ±	0.9	1.30 ±	1.0	1.33 ±	0.9	P < 0.01
Carbohydrates (g/kg)	4.13 ±	2.2	3.99 ±	2.2	4.18 ±	2.2	NS
Fat (g/kg)	0.74 ±	0.4	0.75 ±	0.4	0.74 ±	0.4	NS

[1] All values are means ± SDs. Gender differences were analyzed by an independent t-test.

[2] Variable significantly greater in men than women.

[3] Variable significantly greater in women than men.

[4] Significant level for t-tests were set to P $\leq$ 0.05.

Table 2 – Daily intake of essential amino acids (EAA).

	Entire Cohort (n = 2295)		Men (n = 578)		Women (n = 1717)		
	Mean	SD	Mean	SD	Mean	SD	P
Histidine (mg/kg)	33.88 ±	24.8	33.07 ±	29.0	34.15 ±	23.2	NS
Isoleucine (mg/kg)	59.86 ±	49.5	58.09 ±	55.3	60.45 ±	47.4	NS
Leucine (mg/kg)	101.78 ±	80.5	99.04 ±	91.0	102.70 ±	76.6	NS
Lysine (mg/kg)	89.35 ±	69.3	86.73 ±	79.6	90.24 ±	65.4	NS
Methionine (mg/kg)	28.41 ±	22.0	27.66 ±	25.7	28.65 ±	20.7	NS
Phenylalanine (mg/kg)	55.25 ±	41.6	53.42 ±	47.3	55.86 ±	39.5	NS
Theronine (mg/kg)	47.73 ±	38.4	46.44 ±	43.8	48.17 ±	36.4	NS
Tryptophan (mg/kg)	14.10 ±	11.5	13.87 ±	13.1	14.18 ±	11.0	NS
Valine (mg/kg)	67.86 ±	54.7	65.54 ±	61.1	68.64 ±	52.3	NS

*** Significance level for t-test was set to $P \leq 0.05$**

Table 3 - Partial correlations of PYY with nutritional and body composition characteristics

	Entire Cohort (n = 2295 - 2280)		Male (n = 578 - 573)		Female (n = 1717 - 1707)	
	r	P	r	P	r	P
Weight (kg)	0.03	NS	-0.02	NS	-0.01	NS
Height(cm)	0.04	NS	0.00	NS	0.02	NS
BMI (kg/m^2)	0.02	NS	-0.01	NS	-0.02	NS
Waist (cm)	0.03	NS	-0.01	NS	0.00	NS
Hip (cm)	0.02	NS	-0.04	NS	-0.01	NS
Waist-Hip Ratio	0.04	NS	0.04	NS	0.00	NS
Body fat (%)	0.06	NS	0.01	NS	0.04	NS
Trunk fat (%)	0.05	NS	0.00	NS	0.03	NS
Andriod fat (%)	0.04	NS	0.05	NS	0.00	NS
Gynoid fat (%)	-0.06	0.01	0.03	NS	-0.07	NS
Protein (g/kg)	-0.04	NS	-0.11	0.02	0.00	NS
Carbohydrates (g/kg)	-0.03	NS	-0.06	NS	-0.01	NS
Fat (g/kg)	0.03	NS	0.03	NS	0.04	NS

*** Significance level for partial correlations was set to $P \leq 0.05$**

Table 4 - Partial correlations of PYY with nutritional and body composition characteristics regarding mediation use

	Male				Female			
	NON-Med (n = 357)		Med (n = 209)		NON-Med (n = 692)		Med (n = 1004)	
	r	P	r	P	r	P	r	P
Weight (kg)	0.01	NS	-0.05	NS	0.03	NS	-0.02	NS
Height(cm)	0.00	NS	0.01	NS	0.04	NS	0.01	NS
BMI (kg/m^2)	0.01	NS	-0.06	NS	0.01	NS	-0.01	NS
Waist (cm)	0.01	NS	-0.01	NS	0.02	NS	0.03	NS
Hip (cm)	0.00	NS	-0.09	NS	0.04	NS	-0.02	NS
Body fat (%)	0.01	NS	-0.03	NS	0.02	NS	-0.03	NS
Trunk fat (%)	0.01	NS	-0.04	NS	0.03	NS	-0.01	NS
Andriod fat (%)	0.04	NS	-0.04	NS	0.03	NS	-0.01	NS
Gynoid fat (%)	0.03	NS	-0.03	NS	0.02	NS	-0.08	NS
Histidine (mg/kg)	-0.14	0.01	0.02	NS	0.00	NS	0.00	NS
Isoleucine (mg/kg)	-0.15	0.01	0.01	NS	0.00	NS	-0.02	NS
Leucine (mg/kg)	-0.15	0.00	0.00	NS	0.00	NS	-0.01	NS
Lysine (mg/kg)	-0.15	0.01	0.01	NS	-0.01	NS	-0.01	NS
Methionine (mg/kg)	-0.15	0.01	0.02	NS	-0.01	NS	0.00	NS
Phenylalanine (mg/kg)	-0.15	0.00	0.01	NS	0.00	NS	-0.02	NS
Threonine (mg/kg)	-0.14	0.01	0.01	NS	0.00	NS	-0.01	NS
Tryptophan (mg/kg)	-0.14	0.01	0.01	NS	0.01	NS	0.00	NS
Valine (mg/kg)	-0.15	0.00	0.00	NS	0.00	NS	-0.01	NS

* **Significance level for partial correlations was set to $P \leq 0.05$**

A3.5 - Fasting Serum Peptide YY (PYY) Concentration is Associated with Glucose and Insulin Resistance in pre-menopausal Women.

Cahill,F.,Wadden,D., Gulliver,W., Randell,E., Vasdev,S., Sun, G.(2011) 29th Annual Obesity Society Scientific MeetingABSTRACTID:517-P

Background: The appetite suppressing gut hormone PYY has been suggested to influence insulin sensitivity. However data at the population level is missing. **Objective:** Our objective was to investigate the relationship between PYY with glucose (GLU), insulin (INS) and insulin resistance (IR) in the Newfoundland population. **Design:** 2077 CODING study subjects (Male=595 Female=1573) participated in this study. PYY was measured by enzyme-linked immunosorbent assay (ELISA). Lx20 and immunoassay analyzers measured GLU and INS, respectively. The homeostatic model assessment (HOMA) method was used to quantify IR. Partial correlations (controlling for age and physical activity) were used to determine associations between PYY with GLU, INS and IR. Analyses were also done according to menopausal, smoking, and medication status. ANOVAs were used to evaluate PYY within and between groups. All subjects fasted 12 hr before blood drawings and DXA scans. **Results:** Men have higher PYY levels than women (p=0.001). PYY was positively correlated with GLU (r = 0.09 P=0.006), INS (r = 0.10 P=0.002), IR (r = 0.11; P=0.002) in pre-menopausal women. PYY was found to be positively correlated with GLU (r = 0.10 P=0.01), INS (r = 0.14 P=0.001), IR (r = 0.15; P=0.001), INS and IR in obese men and in normal and obese women. **Conclusion:** Our study suggests that PYY is involved in insulin resistance and glucose regulation among pre-menopausal women. Due to the fact that the associations between PYY with insulin resistance and glucose regulation were gender dependent and adiposity status dependent, further study is warranted.

Table 1 – Biochemical and body composition characteristics.

	Entire Cohort (n = 2077 - 2065)		Male (n = 595 - 583)		Female (n = 1573 - 1559)		
	Mean	SD	Mean	SD	Mean	SD	P
Age (y) [3]	42.88 ±	12.8	40.40 ±	14.4	43.68 ±	12.1	P < 0.001
Weight (kg) [2]	73.67 ±	15.9	85.76 ±	15.3	69.81 ±	14.1	P < 0.001
Height(cm) [2]	165.53 ±	8.4	175.88 ±	6.5	162.22 ±	5.9	P < 0.001
BMI (kg/m^2) [2]	26.56 ±	5.0	27.58 ±	4.5	26.23 ±	5.2	P < 0.001
Waist (cm) [2]	92.15 ±	14.5	97.70 ±	13.9	90.37 ±	14.3	P < 0.001
Hip (cm) [3]	101.31 ±	11.7	100.17 ±	10.3	101.68 ±	12.1	P < 0.02
Body fat (%) [3]	35.00 ±	9.1	25.77 ±	7.6	37.95 ±	7.3	P < 0.001
Trunk fat (%) [3]	37.23 ±	9.3	30.76 ±	8.9	39.30 ±	8.5	P < 0.001
Andriod fat (%) [3]	42.56 ±	10.8	37.18 ±	10.8	44.28 ±	10.3	P < 0.001
Gynoid fat (%) [3]	41.13 ±	9.6	29.15 ±	7.6	44.96 ±	6.5	P < 0.001
Glucose (mmol/L)[2]	5.10 ±	0.8	5.26 ±	0.8	5.04 ±	0.8	P < 0.001
Imsulin (pmol/L)[2]	67.08 ±	43.7	72.31 ±	52.2	65.40 ±	40.5	P < 0.001
Homa_IR[2]	2.27 ±	1.8	2.47 ±	1.9	2.20 ±	1.7	P < 0.001
Homa_β[3]	133.30 ±	110.7	129.43 ±	128.8	134.54 ±	104.2	P < 0.001
Peptide YY (pg/ml) [2]	112.81 ±	74.4	121.63 ±	76.5	109.99 ±	73.6	P < 0.005

[1] All values are means ± SDs. Gender differences were analyzed by an independent t-test.

[2] Variable significantly greater in men than women.

[3] Variable significantly greater in women than men.

[4] Significant level for t-tests were set to $P \leq 0.05$.

Table 2 - Partial correlations of PYY with biochemical and body composition characteristics

	Female					
	Entire Cohort		PreM		PostM	
	r	P	r	P	r	P
Weight (kg)	0.01	NS	0.00	NS	0.03	NS
Height(cm)	0.02	NS	0.02	NS	0.02	NS
BMI (kg/m^2)	0.00	NS	-0.01	NS	0.02	NS
Waist (cm)	0.03	NS	0.01	NS	0.07	NS
Hip (cm)	0.01	NS	0.02	NS	0.01	NS
Body fat (%)	0.00	NS	0.00	NS	0.01	NS
Trunk fat (%)	0.01	NS	0.00	NS	0.03	NS
Andriod fat (%)	0.02	NS	0.01	NS	0.03	NS
Gynoid fat (%)	-0.03	NS	-0.02	NS	-0.04	NS
Glucose (mmol/L)	0.06	0.01	0.09	0.01	0.04	NS
Insulin (pmol/L)	0.10	0.00	0.10	0.00	0.08	0.05
Homa IR	0.10	0.00	0.11	0.00	0.08	0.05
Homa β	0.06	0.02	0.06	NS	0.05	NS

*** Significance level for partial correlations was set to $P \leq 0.05$**

Table 3 - Partial correlations of PYY with biochemical and body composition characteristics among normal-weight, overweight and obese.

	Male						Female					
	Normal Weight		Overweight		Obese		Normal Weight		Overweight		Obese	
	r	P	r	P	r	P	r	P	r	P	r	P
Weight (kg)	-0.05	NS	0.14	NS	-0.07	NS	0.00	NS	-0.03	NS	0.02	NS
BMI (kg/m^2)	-0.04	NS	0.08	NS	-0.05	NS	-0.02	NS	-0.05	NS	0.02	NS
Body fat (%)	-0.04	NS	0.07	NS	-0.05	NS	-0.08	NS	0.02	NS	0.02	NS
Trunk fat (%)	-0.02	NS	0.02	NS	-0.05	NS	-0.04	NS	0.06	NS	0.02	NS
Andriod fat (%)	0.02	NS	0.04	NS	-0.05	NS	0.01	NS	0.05	NS	-0.02	NS
Gynoid fat (%)	-0.03	NS	0.11	NS	-0.07	NS	-0.09	NS	-0.02	NS	-0.06	NS
Glucose (mmol/L)	-0.04	NS	-0.10	NS	0.00	NS	-0.01	NS	0.04	NS	0.10	0.01
Insulin (pmol/L)	-0.01	NS	0.08	NS	0.20	0.00	0.19	0.00	0.00	NS	0.14	0.00
Homa IR	-0.01	NS	0.06	NS	0.17	0.02	0.17	0.00	0.01	NS	0.15	0.00
Homa β	0.02	NS	0.15	NS	0.19	0.00	0.18	0.00	-0.02	NS	0.05	NS

* Significance level for partial correlations was set to $P \leq 0.05$

A3.6 - Association Between the Body Adiposity Index (BAI) and Cardiometabolic Risk Factors (CRFs) among normal-weight (NW), overweight (OW), and obese (OB) men and women in the CODING study.

Cahill, F.,Wadden, D., Amini, P., Lee, A., Yi, Y., Randell, E., Vasdev, S., Sun, G. (2012) 30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374824

Background: The Body Adiposity Index (BAI) is a newly proposed method, developed using Mexican-Americans, to predict adiposity. Although BAI has demonstrated some advantages over Body Mass Index (BMI) as a predictor of whole adiposity, there is very little data regarding its association with Cardiometabolic Risk Factors (CRFs) in Caucasian populations. **Objective:** The purpose of this investigation was to evaluate the relationship between BAI with CRFs and compare the predictive power of both BMI and BAI with CRFs among normal-weight (NW),overweight (OW) and obese (OB).**Design:** 2876 CODING study subjects (Male=734 Female=2052) All subjects fasted 12 hr before blood drawings and DXA scans. Anthropometric (height, weight, waist circumference, hip circumference), and biochemical (glucose, insulin, triglycerides, and LDL cholesterol) makers were measured. Body composition (%BF, %TF, %AF, and %GF) were measured by dual-energy X-ray absorptiometry (DXA). Insulin resistance and β-cell function were determined with the homeostasis model assessment (HOMA-IR, HOMA-β). Pearson correlations, from the entire cohort, along with men and women separately, were employed to compare the correlation of both BAI and BMI with CRFs. In addition, the correlation of both BAI and BMI with CRFs were measured among NW, OW, and OB groups. Adiposity status was determined by the Bray Criteria according to %BF.**Results:** BAI was positively associated with glucose, insulin, HOMA-IR, and triglycerides with higher correlation coefficients in men than BMI. However, glucose, insulin, HOMA-IR, and triglycerides had higher correlation coefficients with BMI than BAI in women. The correlation coefficients between glucose, insulin, HOMA-IR, and triglycerides with BAI remained stronger for men then with BMI after stratified into NW, OW and OB groups. The correlation coefficients between

glucose, insulin, HOMA-IR, and triglycerides with BMI remained stronger for women then with BMI after stratified into adiposity groups.BAI showed higher correlation coefficients with cardiometabolic risk factors in men while BMI had higher correlation with cardiometabolic risk factors in women. **Conclusion:** Our data would suggest that both BMI and BAI have gender specific biases regarding their ability to predict CRFs in the Caucasian population.

Table 1 – Physical and biochemical characteristics of normal-weight, overweight and obese men and women.

	Entire Cohort				Male				Female			
	Normal	Overweight	Obese		Normal	Overweight	Obese		Normal	Overweight	Obese	
	664 - 928	**693 - 818**	**933 - 1040**		**175 - 266**	**162 - 190**	**249 - 278**		**489 - 662**	**531 - 628**	**684 - 762**	
Age (yr)[2,3]	40.4 ± 14.0	44.9 ± 12.3	43.7 ± 12.0	1v2&3	37.1 ± 15.1	44.1 ± 13.4	40.8 ± 13.1	1v2v3	41.8 ± 13.3	45.1 ± 12.0	44.8 ± 11.4	1v2&3
Weight (kg) [2]	64.7 ± 10.7	70.9 ± 11.3	85.3 ± 16.1	1v2v3	76.4 ± 8.9	84.5 ± 9.1	96.2 ± 15.7	1v2v3	60.0 ± 7.2	66.8 ± 8.2	81.4 ± 14.4	1v2v3
Height (cm) [3]	167.1 ± 8.7	165.3 ± 8.2	165.9 ± 9.1	1v2&3	177.0 ± 6.5	175.9 ± 5.8	176.6 ± 7.2	NS	163.2 ± 5.9	162.0 ± 5.7	162.1 ± 6.1	1v2&3
Waist (cm) [2]	82.4 ± 9.0	90.4 ± 10.0	102.8 ± 14.2	1v2v3	87.6 ± 7.9	96.5 ± 7.4	107.5 ± 13.4	1v2v3	80.3 ± 8.5	88.6 ± 10.0	101.1 ± 14.1	1v2v3
Hip (cm) [2]	92.3 ± 6.9	99.3 ± 7.1	110.0 ± 11.5	1v2v3	93.1 ± 6.5	99.0 ± 6.5	107.1 ± 9.8	1v2v3	92.0 ± 7.1	99.4 ± 7.3	111.0 ± 11.9	1v2v3
BMI (kg/m^2) [2]	23.1 ± 2.6	25.9 ± 2.9	30.9 ± 4.9	1v2v3	24.4 ± 2.5	27.3 ± 2.6	30.8 ± 4.4	1v2v3	22.5 ± 2.4	25.4 ± 2.9	31.0 ± 5.1	1v2v3
BAI [2]	24.9 ± 4.3	28.9 ± 4.6	33.7 ± 6.8	1v2v3	21.6 ± 3.1	24.5 ± 3.2	27.8 ± 4.6	1v2v3	26.2 ± 4.0	30.3 ± 4.0	35.9 ± 6.1	1v2v3
Percent Body Fat (%BF) [2]	26.4 ± 6.7	34.8 ± 5.6	42.0 ± 6.6	1v2v3	17.5 ± 4.0	25.4 ± 2.1	33.1 ± 4.0	1v2v3	30.0 ± 3.5	37.7 ± 2.1	45.3 ± 3.6	1v2v3
Percent Trunk Fat (%TF) [2]	27.6 ± 6.6	37.5 ± 4.9	45.0 ± 5.7	1v2v3	21.0 ± 5.6	31.3 ± 3.6	38.8 ± 4.2	1v2v3	30.3 ± 4.8	39.4 ± 3.4	47.2 ± 4.3	1v2v3
Percent Android Fat (%AF) [2]	31.8 ± 8.4	43.0 ± 6.1	51.0 ± 6.2	1v2v3	25.9 ± 8.3	37.8 ± 5.4	45.6 ± 5.3	1v2v3	34.1 ± 7.3	44.6 ± 5.3	53.0 ± 5.2	1v2v3
Percent Gynoid Fat (%GF) [2]	34.4 ± 9.0	40.8 ± 7.9	46.4 ± 7.9	1v2v3	22.4 ± 6.0	28.1 ± 4.0	35.6 ± 4.8	1v2v3	39.3 ± 4.1	44.7 ± 3.6	50.4 ± 4.3	1v2v3
Insulin (pmol/L) [2]	52.0 ± 56.6	64.8 ± 70.1	85.7 ± 71.0	1v2v3	49.3 ± 32.3	68.9 ± 43.5	94.1 ± 79.7	1v2v3	52.9 ± 63.1	63.6 ± 76.4	82.7 ± 67.4	1v2v3
HOMA-IR [2,4]	1.7 ± 3.2	2.2 ± 3.4	3.0 ± 3.3	1v2v3	1.6 ± 1.0	2.4 ± 1.6	3.5 ± 4.9	3v2&1	1.8 ± 3.7	2.1 ± 3.8	2.9 ± 2.4	3v2&1
HOMA-β [4]	112.6 ± 166.5	119.4 ± 256.6	155.5 ± 195.7	3v2&1	92.5 ± 73.0	112.9 ± 81.9	160.9 ± 168.6	3v2&1	119.7 ± 188.6	121.4 ± 289.7	153.5 ± 204.8	3v2&1
TG (mmol/L) [2,3]	1.0 ± 0.6	1.2 ± 0.8	1.4 ± 0.9	1v2v3	1.0 ± 0.6	1.5 ± 1.0	1.7 ± 1.1	1v2&3	1.0 ± 0.5	1.1 ± 0.7	1.3 ± 0.8	1v2v3
Glucose (mmol/L) [2,3]	4.9 ± 0.6	5.1 ± 0.8	5.3 ± 1.0	1v2v3	5.1 ± 0.6	5.3 ± 0.7	5.4 ± 1.2	1v2&3	4.9 ± 0.6	5.0 ± 0.8	5.2 ± 0.9	1v2v3
HDL-C (mmol/L) [2,4]	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	1v2v3	1.3 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	3v2&1	1.6 ± 0.4	1.5 ± 0.4	1.4 ± 0.3	1v2v3
LDL-C (mmol/L) [3]	2.9 ± 0.9	3.2 ± 0.9	3.2 ± 0.9	1v2&3	2.8 ± 0.9	3.3 ± 1.0	3.2 ± 0.9	1v2&3	2.9 ± 0.8	3.2 ± 0.9	3.2 ± 0.9	1v2&3
Systolic BP (mmhg) [2,3]	120.9 ± 15.8	124.6 ± 16.5	127.4 ± 17.0	1v2v3	128.8 ± 13.4	133.5 ± 14.8	134.8 ± 16.7	1v2&3	117.9 ± 15.6	121.9 ± 16.1	124.7 ± 16.4	1v2v3
Diastolic BP (mmhg) [2,3]	78.1 ± 10.3	81.0 ± 11.0	83.7 ± 11.3	1v2v3	79.7 ± 9.2	84.2 ± 10.2	86.7 ± 11.9	1v2&3	77.4 ± 10.6	80.0 ± 11.1	82.6 ± 10.9	1v2v3

[1] All values are means ± SDs. NW, normal-weight; OW, overweight; OB, obese. Subjects were classified on the basis of percentage body fat as normal weight, overweight, or obese according to criteria recommended by Bray . Adiposity status was analyzed by one-way ANOVA. [2] 1v2v3 (1 = NW, 2 = OW, 3 = OB) = OB different then NW and OW with OW different NW. [3] 1v2&3 (1 = NW, 2 = OW, 3 = OB) = NW different then OW and OB. [4] 3v2&1 (1 = NW, 2 = OW, 3 = OB) = OB different then NW and OW . [5] Significant level for one-way ANOVA was set to $P \leq 0.05$.

Table 2 – Pearson correlations of Body Adiposity Index (BAI) & Body Mass Index (BMI) with Cardiometabolic Risk Factors (CRFs)

BMI	Entire Cohort						Male						Female					
	Normal Weight		Overweight		Obese		Normal Weight		Overweight		Obese		Normal Weight		Overweight		Obese	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Percent Body Fat (%BF)	-0.04	NS	-0.10	0.00	0.34	0.00	0.43	0.00	0.27	0.00	0.47	0.00	0.46	0.00	0.44	0.00	0.62	0.00
Percent Trunk Fat (%TF)	0.15	0.00	0.12	0.00	0.35	0.00	0.48	0.00	0.33	0.00	0.41	0.00	0.51	0.00	0.50	0.00	0.47	0.00
Percent Android Fat (%AF)	0.22	0.00	0.18	0.00	0.43	0.00	0.37	0.00	0.23	0.00	0.50	0.00	0.46	0.00	0.41	0.00	0.51	0.00
Percent Gynoid Fat (%AF)	-0.24	0.00	-0.32	0.00	0.15	0.00	0.11	NS	-0.08	NS	0.21	0.00	0.04	NS	-0.21	0.00	0.27	0.00
Waist circumference (cm)	0.69	0.00	0.68	0.00	0.74	0.00	0.69	0.00	0.56	0.00	0.70	0.00	0.64	0.00	0.67	0.00	0.77	0.00
Hip circumference (cm)	0.56	0.00	0.59	0.00	0.78	0.00	0.52	0.00	0.44	0.00	0.73	0.00	0.60	0.00	0.66	0.00	0.80	0.00
Insluin (pmol/L)	0.22	0.00	0.29	0.00	0.40	0.00	0.07	NS	0.27	0.00	0.34	0.00	0.30	0.00	0.29	0.00	0.43	0.00
HOMA-IR	0.29	0.00	0.34	0.00	0.43	0.00	0.11	NS	0.29	0.00	0.34	0.00	0.36	0.00	0.34	0.00	0.46	0.00
HOMA-β	-0.05	NS	0.03	NS	0.20	0.00	-0.11	NS	0.13	NS	0.20	0.00	0.04	NS	0.04	NS	0.20	0.00
TG (mmol/L)	0.20	0.00	0.30	0.00	0.21	0.00	0.20	0.00	0.27	0.00	0.20	0.00	0.22	0.00	0.26	0.00	0.22	0.00
Glucose (mmol/L)	0.37	0.00	0.31	0.00	0.24	0.00	0.20	0.00	0.18	0.01	0.14	0.02	0.40	0.00	0.30	0.00	0.28	0.00
HDL-C (mmol/L)	-0.25	0.00	-0.30	0.00	-0.24	0.00	-0.11	NS	-0.21	0.00	-0.23	0.00	-0.15	0.00	-0.23	0.00	-0.28	0.00
LDL-C (mmol/L)	0.14	0.00	0.14	0.00	-0.03	NS	0.18	0.00	0.11	NS	-0.03	NS	0.15	0.00	0.13	0.00	-0.02	NS
Systolic Blood Pressure (mmhg)	0.32	0.00	0.32	0.00	0.20	0.00	0.28	0.00	0.21	0.01	0.23	0.00	0.23	0.00	0.27	0.00	0.20	0.00
Diastolic Blood Pressure (mmhg)	0.21	0.00	0.26	0.00	0.18	0.00	0.20	0.00	0.11	NS	0.21	0.00	0.18	0.00	0.26	0.00	0.18	0.00

BAI	Entire Cohort						Male						Female					
	Normal Weight		Overweight		Obese		Normal Weight		Overweight		Obese		Normal Weight		Overweight		Obese	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Percent Body Fat (%BF)	0.65	0.00	0.66	0.00	0.69	0.00	0.52	0.00	0.45	0.00	0.46	0.00	0.51	0.00	0.51	0.00	0.56	0.00
Percent Trunk Fat (%TF)	0.65	0.00	0.67	0.00	0.63	0.00	0.53	0.00	0.45	0.00	0.39	0.00	0.50	0.00	0.51	0.00	0.45	0.00
Percent Android Fat (%AF)	0.57	0.00	0.58	0.00	0.62	0.00	0.42	0.00	0.43	0.00	0.45	0.00	0.48	0.00	0.43	0.00	0.47	0.00
Percent Gynoid Fat (%AF)	0.48	0.00	0.45	0.00	0.56	0.00	0.14	0.02	-0.04	NS	0.19	0.00	0.17	0.00	-0.08	NS	0.25	0.00
Waist circumference (cm)	0.27	0.00	0.30	0.00	0.48	0.00	0.61	0.00	0.63	0.00	0.61	0.00	0.55	0.00	0.61	0.00	0.74	0.00
Hip circumference (cm)	0.65	0.00	0.67	0.00	0.80	0.00	0.73	0.00	0.76	0.00	0.80	0.00	0.81	0.00	0.78	0.00	0.87	0.00
Insluin (pmol/L)	0.17	0.00	0.19	0.00	0.29	0.00	0.13	NS	0.34	0.00	0.40	0.00	0.19	0.00	0.26	0.00	0.38	0.00
HOMA-IR	0.19	0.00	0.20	0.00	0.30	0.00	0.17	0.02	0.38	0.00	0.40	0.00	0.23	0.00	0.30	0.00	0.41	0.00
HOMA-β	0.08	0.04	0.12	0.00	0.15	0.00	-0.01	NS	0.16	0.04	0.26	0.00	0.00	NS	0.07	NS	0.15	0.00
TG (mmol/L)	0.19	0.00	0.10	0.01	0.08	0.01	0.26	0.00	0.23	0.00	0.26	0.00	0.20	0.00	0.25	0.00	0.18	0.00
Glucose (mmol/L)	0.15	0.00	0.15	0.00	0.15	0.00	0.21	0.00	0.26	0.00	0.15	0.01	0.28	0.00	0.27	0.00	0.27	0.00
HDL-C (mmol/L)	0.16	0.00	0.11	0.00	0.08	0.01	-0.01	NS	0.04	NS	-0.11	NS	-0.05	NS	-0.13	0.00	-0.17	0.00
LDL-C (mmol/L)	0.23	0.00	0.13	0.00	0.01	NS	0.23	0.00	0.18	0.01	0.00	NS	0.24	0.00	0.20	0.00	0.04	NS
Systolic Blood Pressure (mmhg)	0.02	NS	-0.01	NS	0.02	NS	0.20	0.00	0.30	0.00	0.17	0.01	0.20	0.00	0.17	0.00	0.20	0.00
Diastolic Blood Pressure (mmhg)	0.08	0.02	0.04	NS	0.05	NS	0.24	0.00	0.28	0.00	0.20	0.00	0.12	0.00	0.14	0.00	0.15	0.00

* **Significance level for Pearson correlations was set to P ≤ 0.05**

A3.7 - The Response of Five Functionally Connected Gut Hormones (PP, PYY, CCK, GLP-1 and Ghrelin) to a Short-term Positive Energy Challenge.

Cahill, F.,Wadden, D., Amini, P., Lee, A., Yi, Y., Randell, E., Sun, G. (2012) 30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1375761

Background: The gastrointestinal tract (Gut) secretes a number of hormones which regulate metabolism and appetite through the gut-brain axis. Theoretically gut hormones are functionally associated. However, few studies have investigated whether gut hormones together and how they work as a functionally clustered hormonal group. **Objective:** The purpose of our study was to evaluate how 5 functionally associated gut hormones: PP, PYY, CCK, GLP-1 and Ghrelin respond to a short term positive energy challenge among normal-weight (NW),overweight (OW) and obese (OB) young men.**Design:** The 7-day positive energy challenge was set to 70% above normal energy requirements. The 5 gut hormones were measured using an ELISA method. The NW(8-20.9%), OW(21-25.9%), and OB(>26%) groups were classified by the Bray criteria according to percent body fat measured by dual-emission X-ray absorptiometry (DXA). Partial correlations (controlling for age and %BF) were used to assess the relationships among the 5 gut hormones at baseline and after a 7-day positive energy challenge. **Results:** All 5 gut hormones were positively correlated with one another at baseline (Table 2.) Interesting baseline CCK level is positively correlated with the increase of PYY and GLP-1, which may represent a beneficial joint response to cope with the challenge of overfeeding. PYY at baseline predicts a decreased level of GLP-1 after overfeeding and CCK predicts an increased level of both PP and GLP-1. The 5 gut hormones at baseline were found positively correlated with each other. **Conclusion:** PYY level at baseline predicted a reduced GLP-1 response after overfeeding. However CCK level at baseline predicted a double increases of PYY and GLP-1 in response to a short period of overfeeding. Further study is warranted to understand the cluster response of gut hormones as a functional group in the development of human obesity and diabetes.

Table 1 – Physical and biochemical characteristics of normal-weight, overweight and obese young men.

Physical and biochemical characteristics of subjects at baseline and in response to 7-days of overfeeding[1]

	Entire Cohort (n = 69)		Normal Weight (n = 20 - 27)		Overweight (n = 12-14)		Obese (n = 25-28)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Age	23.11 ± 3.1	NA	23.72 ± 3.6	NA	21.97 ± 3.1	NA	23.25 ± 2.6	NA
Height (cm)	179.02 ± 6.3	NA	179.16 ± 6.5	NA	179.62 ± 4.8	NA	178.91 ± 7.1	NA
Weight (kg)[4, 5]	80.92 ± 15.4	83.13 ± 15.8	72.39 ± 9.2	74.53 ± 9.6	77.81 ± 4.3	79.39 ± 4.3	93.01 ± 15.6	95.65 ± 16.0
BMI (kg/m^2)[4, 5]	25.27 ± 4.8	25.96 ± 4.9	22.55 ± 2.6	23.23 ± 2.8	24.13 ± 1.3	24.63 ± 1.5	29.10 ± 4.9	29.93 ± 5.0
Percent body fat[2, 6]	22.41 ± 8.9	22.69 ± 8.5	14.63 ± 3.3	15.38 ± 3.4	22.54 ± 0.8	22.82 ± 1.1	31.51 ± 5.0	31.26 ± 4.7
HDL cholesterol (mmol/L)[5]	1.31 ± 0.3	1.41 ± 0.3	1.37 ± 0.3	1.47 ± 0.3	1.39 ± 0.3	1.43 ± 0.3	1.19 ± 0.2	1.31 ± 0.3
LDL cholesterol (mmol/L)	2.72 ± 0.7	2.75 ± 0.7	2.61 ± 0.7	2.67 ± 0.7	2.82 ± 0.7	2.83 ± 0.9	2.79 ± 0.7	2.79 ± 0.6
Triglycerols (mmol/L)[4, 5]	1.08 ± 0.5	1.44 ± 1.3	0.90 ± 0.3	1.17 ± 0.8	0.92 ± 0.3	1.01 ± 0.5	1.37 ± 0.7	1.91 ± 1.9
Glucose (mmol/L)	0.71 ± 0.0	5.11 ± 0.5	0.69 ± 0.0	5.03 ± 0.5	0.70 ± 0.0	5.09 ± 0.6	0.72 ± 0.1	5.17 ± 0.5
Insulin (pmol/L)[3, 5]	68.54 ± 70.2	86.84 ± 66.0	42.86 ± 23.5	63.99 ± 23.8	69.51 ± 69.2	88.85 ± 86.3	97.00 ± 91.8	111.98 ± 77.1
HOMA-IR[3, 5]	2.38 ± 2.8	2.92 ± 2.4	1.39 ± 0.8	2.09 ± 0.9	2.36 ± 2.7	2.95 ± 2.9	3.51 ± 3.8	3.85 ± 2.9
HOMA-β[3, 5]	114.57 ± 75.1	158.38 ± 105.1	83.32 ± 38.7	125.63 ± 49.4	120.21 ± 74.1	175.91 ± 163.6	147.49 ± 90.6	189.67 ± 103.4
Ghrelin (pg/ml) [5]	459.86 ± 1529.2	509.62 ± 1488.9	686.59 ± 2422.0	670.65 ± 2314.4	274.91 ± 345.9	284.14 ± 330.0	352.03 ± 594.9	494.66 ± 747.3
Peptide YY(pg/ml) [5]	118.94 ± 57.9	136.73 ± 70.1	121.47 ± 60.9	148.44 ± 93.4	109.90 ± 53.8	119.49 ± 38.9	118.93 ± 56.5	136.47 ± 58.3
GLP-1 (pg/ml) [5]	36.84 ± 26.8	42.39 ± 27.0	37.10 ± 34.9	44.22 ± 34.9	31.59 ± 14.6	32.72 ± 14.7	39.12 ± 23.8	45.55 ± 22.9
Pancreatic Peptide (pg/ml)	1358.79 ± 1270.3	1420.95 ± 1190.8	1245.56 ± 1144.9	1484.09 ± 1178.9	1592.34 ± 1425.7	1540.52 ± 1281.6	1433.13 ± 1360.9	1414.89 ± 1221.1
Cholecystokinin (pg/ml)	66.43 ± 56.3	68.64 ± 58.3	74.39 ± 75.9	80.04 ± 76.4	49.44 ± 20.4	55.00 ± 12.4	68.00 ± 42.6	65.00 ± 49.1

[1] All values are mean ± SD. Homeostasis model assessment of insulin resistance (HOMA-IR) and of B cell function (HOMA-β); NA, not applicable. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%). Obesity status and overfeeding response were analyzed by 2-factor mixed-model ANOVA for repeated measures (IBM SPSS Statistics 20).

[2] Significant difference between normal weight, overweight, and obese subjects at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[3] Significant difference between normal weight and obese subjects at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[4] Significant difference between obese and normal weight and obese and overweight, at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[5] Significant difference due to overfeeding, $P < 0.05$ (2-factor mixed-model ANOVA).

[6] Significant overfeeding x adiposity status interaction, $P < 0.05$ (2-factor mixed-model ANOVA with Bonferroni correction).

Table 2 – Partial correlations of 5 gut hormones at baseline and after the positive energy challenge.

Baseline vs Baseline (Control for %BF)

	PYY		PP		GLP1		CCK		Ghrelin	
	r	P	r	P	r	P	r	P	r	P
PYY			0.45	0.00	0.45	0.00	0.27	0.04	0.53	0.00
PP	0.45	0.00			0.41	0.00	0.50	0.00	0.32	0.01
GLP1	0.45	0.00	0.41	0.00			0.48	0.00	0.48	0.00
CCK	0.27	0.04	0.50	0.00	0.48	0.00			0.34	0.01
Ghrelin	0.53	0.00	0.32	0.01	0.48	0.00	0.34	0.01		

Baseline vs Change (Control for %BF)

	PYY-CH		PP-CH		GLP1-CH		CCK-CH		Ghrelin-CH	
	r	P	r	P	r	P	r	P	r	P
PYY			-0.09	NS	-0.27	0.02	-0.05	NS	0.14	NS
PP	0.01	NS			-0.10	NS	-0.29	0.05	-0.13	NS
GLP1	0.08	NS	0.01	NS			0.10	NS	-0.18	NS
CCK	0.03	NS	0.47	0.00	0.26	0.05			0.14	NS
Ghrelin	0.05	NS	-0.01	NS	0.10	NS	-0.11	NS		

* **Significance level for Pearson correlations was set to $P \leq 0.05$**

A3.8 - Pancreatic Polypeptide (PP) Response to a 7-Day Overfeeding in Young Men.

Cahill, F.,Wadden, D., Amini, P., Lee, A., Randell, E., Vasdev, S., Sun, G. (2012) 30th Annual Obesity Society Scientific Meeting. CONTROL ID: 1374895

Background: Pancreatic Peptide, an anorexigenic hormone released from the pancreas, is involved in energy regulation. The administration of pancreatic peptide in humans reduces food intake. However, little is known how pancreatic peptide responds to a short term positive energy challenge. **Objective:** The purpose of our study was to investigate the nutritional regulation of Pancreatic Peptide in response to a 7-day positive energy challenge (PEC) among normal-weight (NW),overweight (OW) and obese (OB) young men.**Design:** 69 young men participated in the study.The 7-day positive energy challenge was set to 70% above normal energy intake. Fasting serum Pancreatic Peptide was measured via enzyme-linked immunosorbent assay (ELISA). Partial correlations were used to explore the relationship between Pancreatic Peptide levels with obesity makers such as weight, body fat percentage (%BF), trunk fat percentage (%TF), body mass index (BMI), triglycerides, HDL-C, LDL-C, glucose, insulin, insulin resistance (HOMA-IR) and β-cell function (HOMA-β) at baseline and after the positive energy challenge.**Results:** The PEC significantly affected body composition, lipids and insulin profiles, but PP levels were not affected (Table 1). Baseline PP was not associated with baseline obesity markers within the entire cohort or among normal-weight overweight, and obese subjects based on %BF measured by DXA (Table 2). However, baseline PP was negatively associated with the increase in %BF(r= -0.318, p=0.016), %TF(r= -0.309, p=0.020) and insulin resistance in response to the PEC. These relationships remained after controlling for obesity status.**Conclusion:** To our knowledge, this is the first study to explore the association between PP and obesity related phenotypes before and after a PEC. Our findings suggest that PP is not significantly affected by our PEC and is not associated with obesity markers independent of adiposity status. However, higher baseline levels

of PP were found to be inversely correlated with a lower increase of body fat and insulin resistance suggesting a protective role of PP when exposed to a PEC.

Table 1 – Physical and biochemical characteristics of normal-weight, overweight and obese young men.

Physical and biochemical characteristics of subjects at baseline and in response to 7-days of overfeeding[1]

	Entire Cohort (n = 69)		Normal Weight (n = 20 - 27)		Overweight (n = 12-14)		Obese (n = 25-28)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Age	23.1 ± 3.1	NA	23.7 ± 3.6	NA	21.9 ± 3.1	NA	23.2 ± 2.6	NA
Height (cm)	179.1 ± 6.0	NA	179.5 ± 6.3	NA	179.3 ± 4.5	NA	179.0 ± 6.8	NA
Weight (kg)[4, 5]	80.9 ± 15.3	83.1 ± 15.8	72.3 ± 9.2	74.5 ± 9.5	77.8 ± 4.2	79.3 ± 4.2	93.0 ± 15.62	95.6 ± 16.0
BMI (kg/m^2)[4, 5]	25.2 ± 4.7	25.9 ± 4.9	22.5 ± 2.6	23.2 ± 2.8	24.1 ± 1.3	24.6 ± 1.4	29.1 ± 4.8	29.9 ± 5.0
Percent body fat[2, 6]	22.4 ± 8.9	22.6 ± 8.4	14.6 ± 3.3	15.3 ± 3.4	22.5 ± 0.8	22.8 ± 1.0	31.5 ± 5.0	31.2 ± 4.7
Percent trunk fat[2, 6]	25.1 ± 9.9	25.5 ± 9.3	16.5 ± 3.6	17.5 ± 3.7	25.3 ± 1.8	25.7 ± 2.2	35.2 ± 5.3	34.8 ± 5.0
Percent android fat[2, 5]	28.9 ± 11.9	29.8 ± 11.7	19.0 ± 4.4	19.8 ± 4.9	28.8 ± 2.5	29.4 ± 2.7	40.9 ± 7.4	41.5 ± 7.1
Percent gynoid fat	27.4 ± 8.8	27.6 ± 8.3	20.4 ± 4.8	20.8 ± 4.3	27.4 ± 1.7	28.2 ± 1.7	36.0 ± 4.6	35.7 ± 4.5
Total cholesterol (mmol/L)[5]	4.5 ± 0.8	4.7 ± 0.8	4.3 ± 0.9	4.6 ± 0.8	4.6 ± 0.8	4.7 ± 1.0	4.5 ± 0.7	4.7 ± 0.7
HDL cholesterol (mmol/L)[5]	1.3 ± 0.2	1.4 ± 0.2	1.3 ± 0.3	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.2	1.1 ± 0.2	1.3 ± 0.2
LDL cholesterol (mmol/L)	2.7 ± 0.6	2.7 ± 0.6	2.6 ± 0.6	2.6 ± 0.6	2.8 ± 0.7	2.8 ± 0.8	2.7 ± 0.6	2.7 ± 0.6
Triglycerols (mmol/L)[4, 5]	1.0 ± 0.5	1.4 ± 1.3	0.9 ± 0.3	1.1 ± 0.7	0.9 ± 0.3	1.0 ± 0.5	1.3 ± 0.7	1.9 ± 1.9
Glucose (mmol/L)	5.1 ± 0.5	5.1 ± 0.5	4.9 ± 0.3	5.0 ± 0.5	5.0 ± 0.3	5.0 ± 0.5	5.2 ± 0.6	5.1 ± 0.5
Insulin (pmol/L)[3, 5]	68.5 ± 70.1	86.8 ± 66.0	42.8 ± 23.5	63.9 ± 23.7	69.5 ± 69.1	88.8 ± 86.3	97.0 ± 91.8	111.9 ± 77.1
HOMA-IR[3, 5]	2.3 ± 2.8	2.9 ± 2.4	1.3 ± 0.7	2.0 ± 0.8	2.3 ± 2.6	2.9 ± 2.9	3.5 ± 3.8	3.8 ± 2.9
HOMA-β[3, 5]	114.5 ± 75.1	158.3 ± 105.0	83.3 ± 38.6	125.6 ± 49.4	120.2 ± 74.0	175.9 ± 163.5	147.4 ± 90.6	189.6 ± 103.4
Pancreatic Peptide (pg/ml)	1358.7 ± 1270.2	1420.9 ± 1190.8	1245.5 ± 1144.8	1484.0 ± 1178.9	1592.3 ± 1425.6	1540.5 ± 1281.5	1433.1 ± 1360.8	1414.8 ± 1221.0

[1] All values are mean ± SD. Homeostasis model assessment of insulin resistance (HOMA-IR) and of B cell function (HOMA-β); NA, not applicable. Subjects were classifed based on adiposity recommendations by Bray as normal weight (8-20.9%), overweight (21-25.9%), or obese (>26%). Obesity status and overfeeding response were analyzed by 2-factor mixed-model ANOVA for repeated measures (IBM SPSS Statistics 20).

[2] Significant difference between normal weight, overweight, and obese subjects at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[3] Significant difference between normal weight and obese subjects at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[4] Significant difference between obese and normal weight and obese and overweight, at baseline, $P < 0.05$ (one-factor ANOVA with Bonferroni correction).

[5] Significant difference due to overfeeding, $P < 0.05$ (2-factor mixed-model ANOVA).

[6] Significant overfeeding x adiposity status interaction, $P < 0.05$ (2-factor mixed-model ANOVA with Bonferroni correction).

Table 2 – Partial correlations of baseline PP concentrations with baseline physical and biochemical characteristics.

	Baseline vs Basline							
	Entire Cohort		Normal Weight		Overweight		Obese	
	r	P	r	P	r	P	r	P
Height (cm)	0.06	NS	-0.10	NS	0.37	NS	0.05	NS
Weight (kg)	-0.07	NS	-0.14	NS	-0.03	NS	-0.10	NS
BMI (kg/m^2)	-0.10	NS	-0.10	NS	-0.40	NS	-0.11	NS
Percent Body Fat (%BF)	-0.01	NS	-0.27	NS	-0.21	NS	-0.06	NS
Percent Trunk Fat (%TF)	0.00	NS	-0.17	NS	-0.03	NS	-0.08	NS
Percent Android Fat (%AF)	-0.02	NS	-0.05	NS	-0.32	NS	-0.16	NS
Glucose (mmol/L)	-0.04	NS	0.02	NS	0.24	NS	-0.05	NS
Total Cholesterol (mmol/L)	-0.15	NS	-0.20	NS	-0.08	NS	-0.09	NS
TG (mmol/L)	-0.03	NS	-0.28	NS	0.43	NS	0.07	NS
HDL-C (mmol/L)	-0.10	NS	-0.29	NS	-0.38	NS	0.07	NS
LDL-C (mmol/L)	-0.11	NS	-0.03	NS	-0.08	NS	-0.12	NS
Insulin (pmol/L)	-0.03	NS	-0.14	NS	0.28	NS	-0.19	NS
HOMA-IR	-0.03	NS	-0.13	NS	0.28	NS	-0.18	NS
HOMA-B	-0.02	NS	-0.21	NS	0.27	NS	-0.20	NS

*** Significance level for Pearson correlations was set to $P \leq 0.05$**

Table 3 – Partial correlations of baseline PP concentrations with changes in physical and biochemical characteristics.

	Baseline vs Change							
	Entire Cohort		Normal Weight		Overweight		Obese	
	r	P	r	P	r	P	r	P
Weight (kg)	0.07	NS	0.04	NS	-0.18	NS	0.25	NS
BMI (kg/m^2)	0.07	NS	0.05	NS	-0.19	NS	0.26	NS
Percent Body Fat (%BF)	-0.29	0.02	-0.26	0.03	-0.20	0.04	-0.39	0.01
Percent Trunk Fat (%TF)	-0.28	0.03	-0.21	0.04	-0.15	NS	-0.40	0.01
Percent Android Fat (%AF)	-0.23	NS	-0.36	NS	-0.33	NS	0.00	NS
Glucose (mmol/L)	-0.13	NS	0.03	NS	-0.34	NS	-0.09	NS
Total Cholesterol (mmol/L)	-0.11	NS	-0.07	NS	-0.14	NS	-0.13	NS
TG (mmol/L)	-0.27	0.04	-0.09	NS	0.17	NS	-0.33	NS
HDL-C (mmol/L)	0.08	NS	0.09	NS	-0.11	NS	-0.13	NS
LDL-C (mmol/L)	0.00	NS	-0.09	NS	-0.16	NS	0.13	NS
Insulin (pmol/L)	-0.31	0.02	-0.11	NS	-0.29	NS	-0.20	NS
HOMA-IR	-0.31	0.02	-0.08	NS	-0.34	NS	-0.19	NS
HOMA-B	-0.30	0.02	-0.21	NS	-0.03	NS	-0.22	NS

*** Significance level for Pearson correlations was set to $P \leq 0.05$**

A3.9 - Significant Association of Dietary Macronutrient Intake with Serum Gastrointestinal Hormones in Obese Children.

Cahill, F.,Wadden, D., Amini, P., Bridger, T., Gulliver, W., Sun, G. (2013) 31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1754611

Background: Gastrointestinal (gut) hormones play an important role in the regulation of food intake and metabolism. Dysregulation of gut hormones are often seen in obese children. However, there is very little data regarding the effect of macronutrient (fat, protein and carbohydrates) intake on circulating levels of gut hormones in childhood obesity. **Objective:** The purpose of this investigation was to evaluate the relationship of the dietary intake of fat, protein and carbohydrates with fasting serum ghrelin, PYY, GLP-1, and Leptin among 100 obese children recruited from the Newfoundland population. **Design:** Circulatingghrelin, PYY, GLP-1, and leptin were measured implementing the Luminex MAGPIX platform employing Milliplex magnetic bead assays. Macronutrient intake was evaluated using the Willet Food Frequency Questionnaire and computed with the Nutribase software suite. **Results:** Stepwise multiple regression analysis was performed to assess the association of dietary macronutrient intake with circulating ghrelin, PYY, GLP-1, and leptin. One-way analysis of variance obese children were ranked and divided into tertiles (Low, Medium, High) based upon dietary intake of carbohydrates, fat, protein to further assess dietary macronutrient intake appetite regulating hormones. Dietary carbohydrate was positively associated with fasting ghrelin (**β** 2.67, **S.E.** 0.71) and negatively associated with GLP-1 (**β** -0.80, **S.E.** 0.30) and leptin (**β** -1.29, **S.E.** 0.48). Dietary fat intake was positively associated with fasting ghrelin (**β** 14.17, **S.E.** 3.93, p = 0.001) and negatively associated with GLP-1 (**β** -3.58, **S.E.** 1.65, p = 0.033) and PYY (**β** -0.26, **S.E.** 0.12, p = 0.033). Higher circulating levels of ghrelin (19.86±12.6 vs. 38.8±30.3pg/ml, P = 0.049) were found in those with high carbohydrate intake. High dietary fat intake was significantly lower levels of PYY (66.27±43.1 vs. 42.43±28.1pg/ml, P = 0.029), GLP1 (18.48±9.2 vs. 13.61±5.8pg/ml, P =

0.038), and leptin (26.98±14.3 vs. 19.67±9.9ng/ml, P = 0.04). **Conclusion:** In summary, our results provide evidence that lowering dietary fat and carbohydrate intakes may help to normalize circulating gut hormones which are associated with lower body fat.

Table 1 - Physical, Biochemical, and Nutritional Characteristics of Obese Children

	Entire Cohort (n = 100)	Male (n = 47)	Female (n = 53)	
	Mean SD	Mean SD	Mean SD	P
Age (y)	11.71 ± 2.8	11.79 ± 2.6	11.64 ± 3.0	**NS**
Height (cm)	155.77 ± 14.8	157.51 ± 14.4	154.26 ± 15.0	**NS**
Weight (kg)	79.26 ± 25.4	80.14 ± 24.7	78.49 ± 26.1	**NS**
BMI (kg/m^2)	31.81 ± 6.2	31.66 ± 6.1	31.93 ± 6.2	**NS**
Body fat (%) [3]	45.60 ± 5.7	43.65 ± 6.2	47.25 ± 4.8	< 0.001
Waist (cm) [2]	104.62 ± 17.8	108.37 ± 16.0	101.65 ± 18.7	< 0.001
Hip (cm)	105.64 ± 17.4	107.10 ± 14.7	104.51 ± 19.3	**NS**
Calories (Kj/Day)	2212.74 ± 984.4	2200.14 ± 963.5	2223.72 ± 1011.2	**NS**
Dietary Protein Intake (g/day)	94.39 ± 41.7	97.48 ± 47.2	91.71 ± 36.5	**NS**
Dietary Protein Intake (g/kg/day)	1.29 0.7	1.28 0.7	1.29 0.6	**NS**
Dietary Carbohydrate Intake (g/day)	326.87 ± 152.7	319.57 ± 140.8	333.22 ± 163.4	**NS**
Dietary Carbohydrate Intake (g/kg/day)	4.50 2.6	4.36 2.3	4.69 2.8	**NS**
Dietary Fat Intake (g/day)	62.20 ± 34.3	62.84 ± 34.8	61.64 ± 34.1	**NS**
Dietary Fat Intake (g/kg/day)	0.84 0.5	0.82 0.4	0.86 0.5	**NS**
Peptide YY (pg/ml)	57.45 ± 39.0	60.34 ± 40.9	55.16 ± 37.6	**NS**
Ghrelin (pg/ml)	28.91 ± 26.4	25.23 ± 14.0	32.48 ± 34.4	**NS**
GLP-1 (pg/ml)	15.89 ± 7.6	15.45 ± 6.9	16.21 ± 8.1	**NS**
Leptin (ng/ml) [3]	24.37 ± 13.2	19.46 ± 10.3	28.19 ± 14.0	< 0.001

[1] All values are means ± SDs. Gender differences were analyzed by a one-way ANOVA.

[2] Variable significantly greater in men than women.

[3] Variable significantly greater in women than men.

[4] Significance level for one-way ANOVA was set to $P \leq 0.05$.

Table 2. Stepwise Multiple Regression of **Carbohydrate Intake (g/kg)** on Appetite Regulating Hormones.

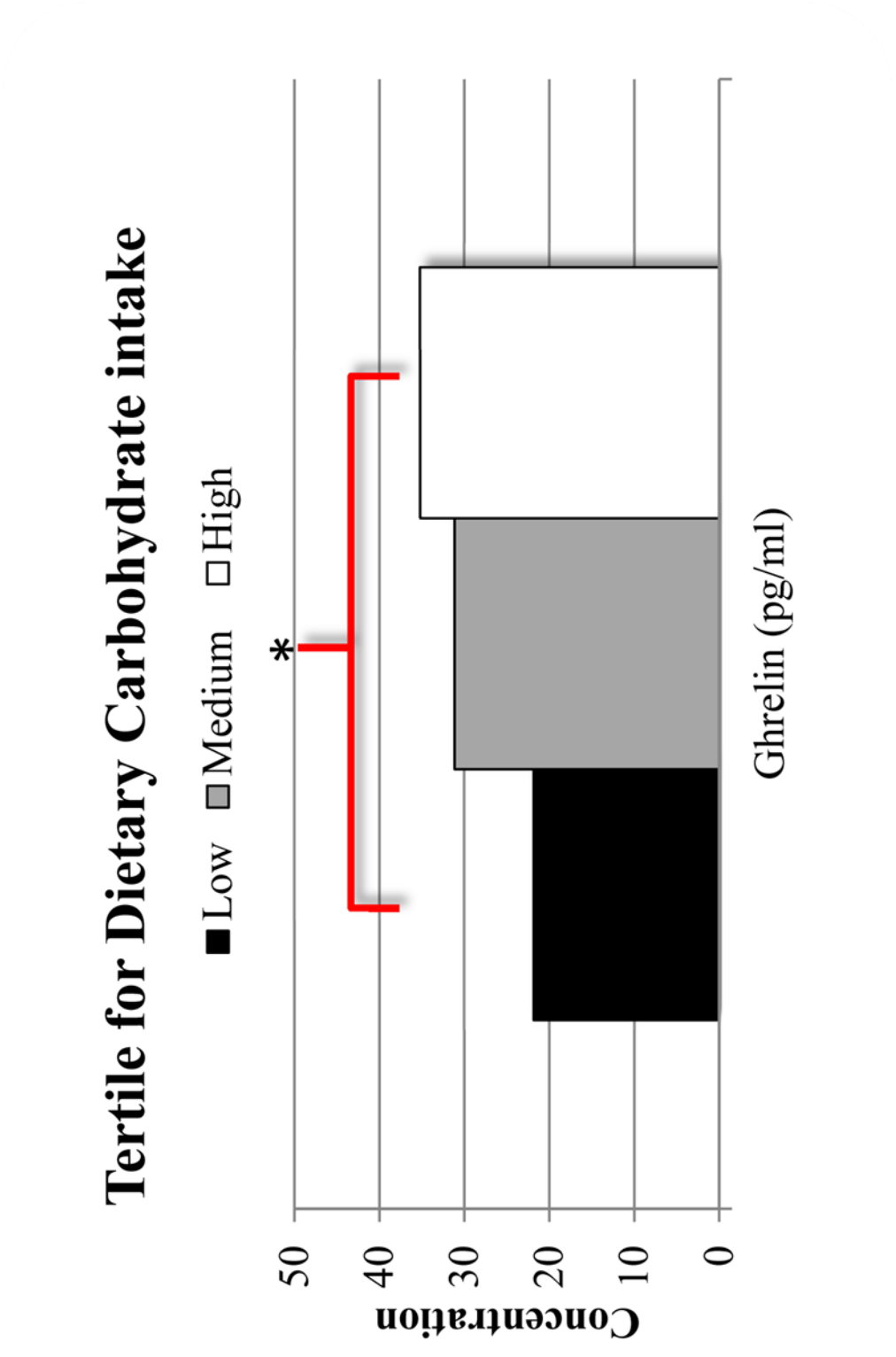
	β	S.E.	β*	P
Ghrelin	2.67	0.71	0.430	≤ 0.001
GLP-1	-0.80	0.30	-0.281	0.008
PYY	-	-	-	-
Leptin	-1.29	0.48	-0.253	0.009

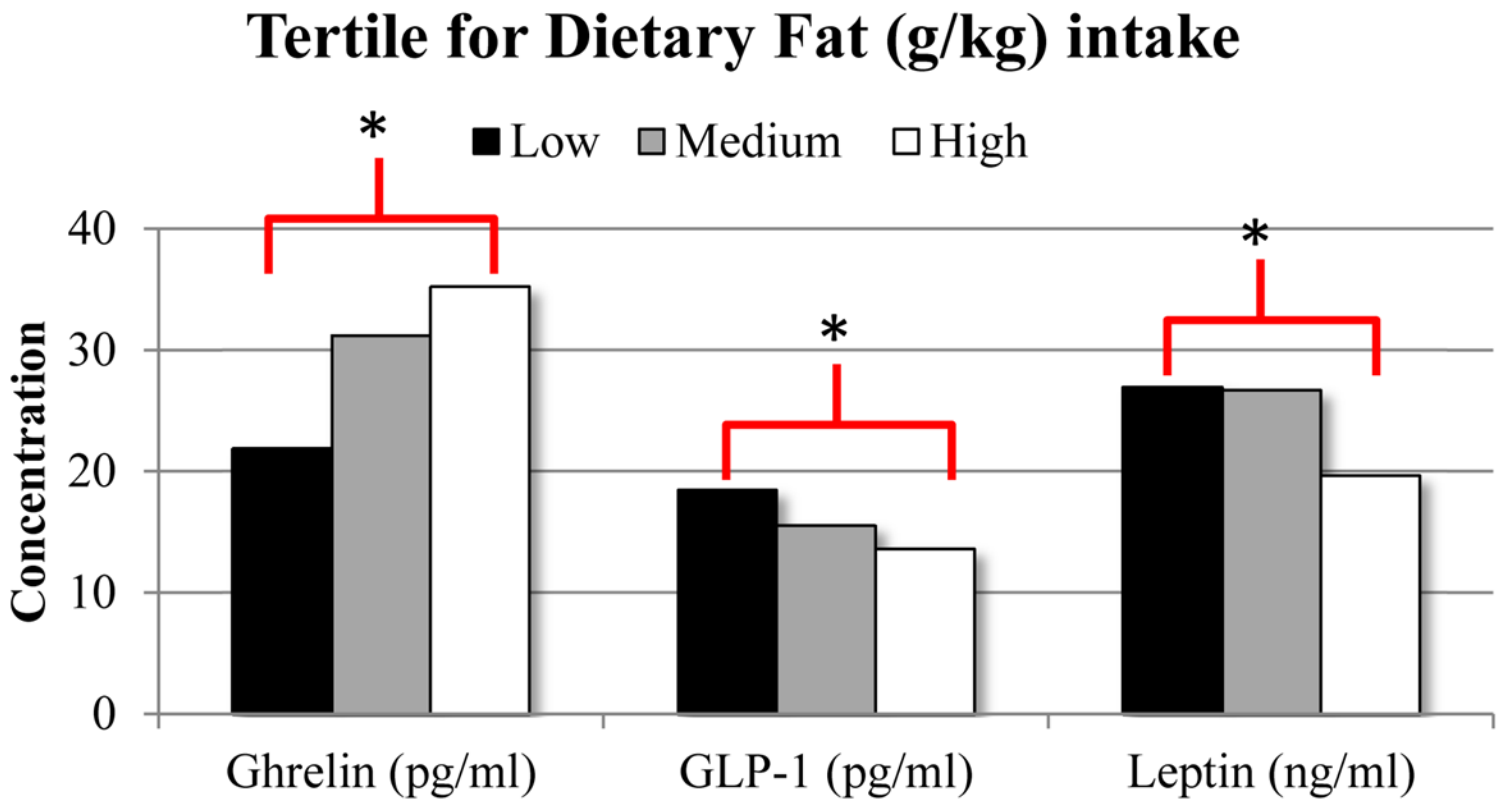
1. Stepwise Multiple Regression model adjusting for age and sex.
2. β = Unstandardized Beta, *β = Standardized Beta
3. Significance level was set to P ≤ 0.05

Table 5. Stepwise Multiple Regression of **Fat Intake (g/kg)** on Appetite Regulating Hormones.

	β	S.E.	β*	P
Ghrelin	14.17	3.93	0.416	0.001
GLP-1	-3.58	1.65	-0.229	0.033
PYY	-0.26	0.12	-0.218	0.033
Leptin	-	-	-	-

1. Stepwise Multiple Regression model adjusting for age and sex.
2. β = Unstandardized Beta, *β = Standardized Beta
3. Significance level was set to P ≤ 0.05


Tertile for Dietary Carbohydrate intake
Low
Medium
High
*
Concentration
50
40
30
20
10
0
Ghrelin (pg/ml)


Tertile for Dietary Fat (g/kg) intake
Low
Medium
High
Concentration
40
30
20
10
0
*
*
*
Ghrelin (pg/ml)
GLP-1 (pg/ml)
Leptin (ng/ml)

A3.10 - The Response and Interaction of Adiponectin, Leptin, and TNF-α Due to a Short-term Positive Energy Challenge.

Cahill,F.,Amini, P., Wadden, D., Ji, Y., Khalili, S., Randell, E., Vasdev, S., Sun, G. (2013)
31st Annual Obesity Society Scientific Meeting. CONTROL ID: 1754590

Background: Adiponectin, leptin, and TNF-α areadipokines which affect, insulin sensitivity, appetite and energy homeostasis. However little data are available regarding the response and interaction of these three adipokines under a positive energy challenge (PEC), which is a major factor in the development of obesity. Therefore, we investigated the response and potential interaction(s) of circulating adiponectin, leptin and TNF-α to a PEC along with the influence of obesity status. **Objective:** The purpose of our study was measure serum adiponectin, leptin, TNF-α before and after 7-days of overfeeding to investigate their response to a positive energy challenge and the functional interaction which may exist between these peptides among normal-weight (NW) and overweight/obese (OW/OB) young men.**Design:** The 7-day positive energy challenge was set to 70% above normal energy requirements. The NW (8-20.9 %BF), OW/OB (>21 %BF) groups were classified by the Bray criteria according to percent body fat measured by dual-emission X-ray absorptiometry (DXA). ANOVA and multiple regression analysis were used to assess the relationships among the three adipokines before and after the 7-days of overfeeding in NW and OW/OB young men. **Results:** Adiponectin and TNF-α at baseline were not associated with body composition. After overfeeding circulating TNF-α increased in NW men only ($p=0.032$) and adiponectin increased ($p<0.001$) independent of adiposity status. Circulating leptin concomitantly increased with adiposity at baseline ($p<0.001$) and also increased in response to the PEC ($p<0.001$). Among OW/OB, baseline TNF-α was negatively associated with the change in adiponectin (β -4.21, S.E. 1.85, $p = 0.028$) while baseline leptin was positively associated with the change in TNF-α (β 0.14, S.E. 0.04, $p<0.001$) due to overfeeding after adjusting for adiposity at baseline. Among OW/OB, the change in TNF-α, due to overfeeding,

was negatively associated with the change in adiponectin (β -1.75, S.E. 0.44, p = 0.01) overfeeding after adjusting for adiposity at baseline. **Conclusion:** Circulating levels of adiponectin, leptin and TNF-α were increased by the PEC. Additionally, our results suggest that baseline levels of TNF-α and leptin predict an attenuated response in adiponectin and an facilitated response of TNF-α ,due to a PEC independent of adiposity, respectively.

Table 1 - Physical and biochemical characteristics of subjects at baseline and in response to 7-days of overfeeding[1]

	Entire Cohort (n = 64)		Normal Weight [2] (n = 25)		Overweight/Obese [2] (n = 39)	
	Baseline	**Overfeeding**	**Baseline**	**Overfeeding**	**Baseline**	**Overfeeding**
Age (y)	23.18 ± 3.2	NA	23.87 ± 3.7	NA	22.74 ± 2.7	NA
Height (cm)	179.31 ± 6.4	NA	178.89 ± 6.6	NA	179.57 ± 6.3	NA
Weight (kg) [3,4,5,6]	80.94 ± 14.3	83.13 ± 14.7	72.26 ± 9.6	74.42 ± 9.6	86.52 ± 14.1	88.71 ± 14.6
BMI (kg/m^2) [3,4,5,6]	25.16 ± 4.0	25.84 ± 4.2	22.58 ± 2.7	23.26 ± 2.9	26.82 ± 3.9	27.50 ± 4.1
Percent Body Fat (%) [3,4,5,6,7]	22.89 ± 8.2	23.20 ± 7.7	14.82 ± 3.4	15.60 ± 3.4	28.06 ± 5.7	28.07 ± 5.5
Percent Trunk Fat (%) [3,4,5,6,7]	25.81 ± 9.1	26.24 ± 8.7	16.78 ± 3.7	17.78 ± 3.8	31.60 ± 6.5	31.67 ± 6.2
Percent Android Fat (%) [3,4,5,6]	29.34 ± 10.6	30.08 ± 10.6	19.38 ± 4.4	20.16 ± 5.0	35.89 ± 8.0	36.60 ± 7.9
Glucose (mmol/L)	5.09 ± 0.5	5.07 ± 0.5	4.98 ± 0.4	5.03 ± 0.5	5.16 ± 0.6	5.11 ± 0.5
Insulin (pmol/L) [3,4,5,6]	59.68 ± 40.35	82.11 ± 57.1	44.22 ± 23.8	64.02 ± 23.7	70.12 ± 45.8	94.33 ± 68.9
HOMA-IR [3,4,5,6]	2.16 ± 2.0	2.88 ± 2.4	1.43 ± 0.8	2.09 ± 0.9	2.64 ± 2.39	3.41 ± 2.9
HOMA-β [3,4,5,6]	107.97 ± 52.29	154.13 ± 90.70	85.45 ± 39.4	126.34 ± 49.9	123.19 ± 54.8	172.91 ± 106.7
Leptin (ng/ml) [3,4,5,6,7]	3.29 ± 2.9	4.64 ± 3.7	1.37 ± 0.9	2.36 ± 1.7	4.56 ± 3.1	6.14 ± 4.0
TNF-Alpha (pg/ml) [5,6,7]	5.55 ± 2.1	5.97 ± 3.3	5.94 ± 2.1	7.17 ± 4.4	5.30 ± 2.0	5.20 ± 2.0
Adiponectin (µg/mL) [4,5]	11.52 ± 5.9	13.70 ± 5.1	11.60 ± 6.3	13.96 ± 4.5	11.47 ± 5.8	13.54 ± 5.5

[1] All values are means ± SDs. HOMA - IR, Homeostasis model assessment of insulin resistance; HOMA – β, Homeostasis model assessment of β cell function; NA, not applicable. Adiposity status and response to overfeeding analyzed by 2 - factor mix model ANOVA (SPSS, version 19.0 Chicago, IL, USA) for repeated measures.

[2] Subjects were classified on the basis of %BF as either Normal Weight (8 - 20.9%) or Overweight/Obese (21% > 26%) according to criteria recommended by Bray [31].

[3] Significant difference between Normal weight, Overweight/Obese subjects at baseline (Student t-test, $P < 0.05$).

[4] Significant difference due to overfeeding within the entire cohort (Student t-test, $P < 0.05$)

[5] Significant difference due to overfeeding (Two-Way mixed model ANOVA, $P < 0.05$).

[6] Significant difference in overfeeding response due to adiposity status (Two-Way mixed model ANOVA, $P < 0.05$).

[7] Significant overfeeding x adiposity status interaction (Two-Way mixed model ANOVA, $P < 0.05$).

[8] Significant difference within group (paired *t*-test, $P < 0.05$).

Table 2. The association of **baseline** adiponectin, TNF-α and leptin with physical and biochemical characteristics at **baseline**.

	Enitre Cohort			Adiposity Status					
				Normal weight			Overweight/Obese		
	β	S.E.	p	β	S.E.	p	β	S.E.	p
Adiponectin									
BMI (kg/m²)									
Unadjusted	-0.170	0.08	0.05	-0.099	0.09	**NS**	-0.216	0.11	**NS**
Adjusted	-0.171	0.09	**NS**	-0.110	0.11	**NS**	-0.246	0.12	**NS**
TF%									
Unadjusted	-0.225	0.19	**NS**	0.008	0.12	**NS**	-0.377	0.17	0.04
Adjusted	-0.142	0.22	**NS**	0.023	0.15	**NS**	-0.329	0.19	**NS**
Insulin (pmol/L)									
Unadjusted	-2.439	0.81	0.00	-0.692	0.78	**NS**	-3.755	1.15	0.00
Adjusted	-1.653	0.86	0.05	-0.244	0.77	**NS**	-3.044	1.38	0.03
HOMA-IR									
Unadjusted	-0.103	0.04	0.02	-0.021	0.03	**NS**	-0.164	0.06	0.01
Adjusted	-0.101	0.05	0.05	-0.008	0.03	**NS**	-0.189	0.08	0.03
HOMA-β									
Unadjusted	-2.865	1.06	0.01	-1.547	1.26	**NS**	-3.849	1.43	0.01
Adjusted	-1.272	1.13	**NS**	-0.414	1.27	**NS**	-2.338	1.73	**NS**
Leptin									
BMI (kg/m²)									
Unadjusted	0.839	0.12	0.00	1.849	0.52	0.00	0.621	0.14	0.00
Adjusted	0.754	0.13	0.00	1.884	0.61	0.01	0.494	0.14	0.00
BF%									
Unadjusted	2.079	0.23	0.00	2.158	0.66	0.00	1.200	0.23	0.00
Adjusted	1.788	0.24	0.00	2.202	0.78	0.01	1.050	0.24	0.00
TF%									
Unadjusted	2.324	0.26	0.00	2.054	0.76	0.01	1.351	0.26	0.00
Adjusted	2.059	0.26	0.00	2.101	0.91	0.03	1.112	0.28	0.00
AF%									
Unadjusted	2.716	0.31	0.00	1.821	0.98	**NS**	1.681	0.33	0.00
Adjusted	2.248	0.32	0.00	1.443	1.13	**NS**	1.331	0.34	0.00
Insulin									
Unadjusted	4.581	1.28	0.00	12.415	5.03	0.02	3.328	1.79	**NS**
Adjusted	2.947	1.35	0.03	12.930	3.71	0.00	1.779	1.88	**NS**
HOMA-IR									
Unadjusted	0.134	0.08	0.01	0.412	0.17	0.02	0.042	0.11	**NS**
Adjusted	0.098	0.09	0.02	0.427	0.13	0.00	0.018	0.13	**NS**
HOMA-β									
Unadjusted	7.636	1.92	0.00	20.420	8.30	0.02	5.672	2.59	0.04
Adjusted	5.762	1.99	0.01	21.492	6.12	0.00	4.213	2.73	**NS**

Table 3. The association of **baseline** adiponectin, TNF-α and leptin concentration with the **change** in physical and biochemical characteristics due to overfeeding.

	Enitre Cohort			Adiposity Status					
				Normal weight			Overweight/Obese		
	β	S.E.	p	β	S.E.	p	β	S.E.	p
TNF-α									
Insulin (pmol/L)									
Unadjusted	4.565	2.15	0.04	2.153	1.89	NS	6.781	3.45	0.05
Adjusted	4.589	2.17	0.04	1.721	1.73	NS	6.474	3.50	NS
HOMA-β									
Unadjusted	7.075	3.85	NS	-1.675	4.00	NS	14.379	5.80	0.02
Adjusted	7.215	3.84	NS	-2.221	3.97	NS	13.178	5.76	0.03
Adiponectin (ng/ml)									
Unadjusted	-0.084	0.20	NS	3.814	6.03	NS	-3.958	1.85	0.05
Adjusted	-0.148	0.17	NS	1.907	4.41	NS	-4.213	1.85	0.03
Leptin									
BMI (kg/m^2)									
Unadjusted	0.039	0.02	0.04	0.084	0.13	NS	0.055	0.02	0.01
Adjusted	0.019	0.03	NS	-0.021	0.16	NS	0.039	0.02	NS
BF%									
Unadjusted	-0.150	0.05	0.00	-0.175	0.30	NS	-0.104	0.05	0.04
Adjusted	-0.054	0.07	NS	-0.092	0.37	NS	-0.047	0.06	NS
TF%									
Unadjusted	-0.236	0.07	0.00	-0.148	0.43	NS	-0.205	0.07	0.01
Adjusted	-0.136	0.10	NS	0.063	0.50	NS	-0.157	0.10	NS
Insulin (pmol/L)									
Unadjusted	2.710	1.55	NS	4.225	4.61	NS	3.326	2.34	NS
Adjusted	3.651	2.39	NS	10.198	4.10	0.02	2.994	2.44	NS
HOMA-IR									
Unadjusted	0.085	0.06	NS	0.194	0.17	NS	0.104	0.09	NS
Adjusted	0.110	0.10	NS	0.379	0.16	0.02	0.109	0.09	NS
TNF-α (pg/ml)									
Unadjusted	0.006	0.10	NS	-0.338	1.02	NS	0.120	0.05	0.03
Adjusted	0.021	0.09	NS	-0.495	0.88	NS	0.145	0.04	0.00

Table 4. The association of the **change** in adiponectin, TNF-α and leptin in response to overfeeding with the **change** in physical and biochemical characteristics due to overfeeding

	Enitre Cohort			Adiposity Status					
				Normal weight			Overweight/Obese		
TNF-α									
Adiponectin (pg/ml)									
Unadjusted	-0.284	0.24	**NS**	-0.290	0.38	**NS**	-0.142	0.36	**NS**
Adjusted	-0.155	0.22	**NS**	-0.509	0.45	**NS**	-1.175	0.44	0.01
Leptin									
BMI (kg/m^2)									
Unadjusted	0.072	0.03	0.02	0.311	0.14	0.04	0.062	0.03	**NS**
Adjusted	0.062	0.04	**NS**	0.339	0.15	0.04	0.048	0.04	**NS**
BF%									
Unadjusted	-0.292	0.09	0.00	0.146	0.54	**NS**	-0.259	0.07	0.00
Adjusted	-0.192	0.10	0.05	0.214	0.62	**NS**	-0.250	0.08	0.00
TF%									
Unadjusted	-0.418	0.13	0.00	0.287	0.77	**NS**	-0.389	0.11	0.00
Adjusted	-0.299	0.14	0.04	0.357	0.87	**NS**	-0.361	0.11	0.00
INSULIN									
Unadjusted	6.015	1.94	0.00	14.756	6.73	0.04	5.706	2.32	0.02
Adjusted	5.971	1.99	0.00	14.377	6.37	0.04	5.570	2.39	0.03
HOMA - IR									
Unadjusted	0.246	0.08	0.00	0.649	0.24	0.02	0.237	0.10	0.02
Adjusted	0.250	0.08	0.00	0.550	0.26	0.05	0.234	0.10	0.02